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RESEARCH PAPERS

Brassica-based seedmeal biofumigation to control *Phytophthora cinnamomi* in the Spanish "*dehesa*" oak trees

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Summary. *Phytophthora cinnamomi* causes a highly destructive root rot that seriously affects oak trees in semi-natural woodlands known as "*dehesa*s". Biofumigation with *Brassica* spp. is a promising tool for disease management. We demonstrated that ground seeds from *B. carinata* and *B. juncea* can inhibit mycelial growth and decrease chlamydospore viability of *P. cinnamomi* in soil. In contrast, *B. napus* seedmeals were ineffective. Reduction of root necrosis in *Lupinus* plants was also achieved when soils were biofumigated with *B. carinata* or *B. juncea* seedmeals. Seedmeal effectiveness was strongly correlated with high sinigrin (2-propenyl glucosinolate) content. We conclude that biofumigation with seedmeals rich in sinigrin could be effective as part of integrated management of oak disease caused by *P. cinnamomi* in "*dehesas*".

Key words: *Brassica carinata*, *Brassica juncea*, *Brassica napus*, sinigrin.

Introduction

There is social rejection of chemical fumigation as a tool to control plant pathogens, mainly due to the associated environmental and human health side effects (Alabouvette, 2006). However, effective alternatives to chemical treatments are not readily available. *Phytophthora cinnamomi* Rands is a commonly-occurring pathogen worldwide, and is responsible for the decimation of native flora in many regions (Brasier, 1996; Hardham, 2005). Currently, holm and cork oaks (*Quercus ilex* L. and *Q. suber* L.) are at risk because of emergence of *P. cinnamomi* in parts of Spain (Sánchez *et al*., 2006). In the Southern Iberian Peninsula, these evergreen oaks grow in agroforestry systems commonly known as "*dehesa*". "*Dehesas*" are semi-natural production systems where chemical fumigation is not acceptable. Biofumigation possibly provides an acceptable and promising alternative to chemical fumigation.

Biofumigation is often based on the toxicity of isothiocyanates (ITCs) released following the hydrolysis of glucosinolates (GSLs) (Kirkegaard *et al*., 1993). GSLs are secondary metabolites produced by plants of the order *Capparales* (Halkier and Gershenzon, 2006) when soils are amended with either fresh plant material or seedmeals (Brown and Morra, 1997). There are more than 130 common GSLs found in *Brassica* spp. (Agerbirk and Olsen, 2012), each documented to have different noxious activity on specific target pathogens. GSL-containing plants have been demonstrated to reduce weeds (Rice *et al*., 2007; Fourie *et al*., 2015), pests (Elberson *et al*., 1997) and soilborne pathogens (Bomford *et al*., 2009; Mazzola *et al*., 2015).

The mechanical incorporation of cover crops into soil is the most common way to perform biofumi-

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gation in agricultural lands. Rainfall or irrigation is needed at the time of plant incorporation in the soil, to ensure effective hydrolysis of GSLs (Morra and Kirkegaard, 2002), as well as efficient breakdown of plant tissues.

Biofumigation based on the incorporation of fresh plant residue in the soil represents a promising tool for the control of root rot caused by *P. cinnamomi* in oaks (Ríos *et al*., 2016a, 2016b). There are factors that may limit biofumigation effectiveness, however, including stoniness hindering effective incorporation of fresh biomass into soil, or lack of water where irrigation may not be feasible.

Cold crushing of seeds for oil removal is routinely performed by the oil and biodiesel industry, and this results in seedmeal rich in GSLs (Morra, 2004). The use of seedmeals as biofumigants also requires wet soil, but the disruption of plant tissue has been previously maximized, and thus GSL hydrolysis may occur rapidly in seedmeals. Furthermore, seedmeal can be applied when the soil is naturally wet, thus synchronizing treatment with the peak of pathogen activity, theoretically increasing the efficiency of disease control (Mazzola *et al*., 2015). While biofumigation using seedmeal represents a possible biofumigation approach in water-limited semi-natural ecosystems, its true viability as a control approach will depend on its efficacy, on the dosage needed to reach efficacy, and on seedmeal availability when and where treatments need to be applied.

The biocidal action of green tissues of some *Brassica* biofumigants against *P. cinnamomi* has been recently reported (Morales-Rodríguez *et al*., 2016; Ríos *et al*., 2016a, 2016b), but biofumigant potential of seeds remains unknown. The aim of the study reported here was to test the effectiveness of ground seeds from three *Brassica* species, *B. napus*, *B. carinata* and *B. juncea*, for control of disease caused by *P. cinnamomi*.

Materials and methods

Seedmeal material

Two genotypes of each of three *Brassica* species were tested for their effectiveness for reducing mycelial growth of *P. cinnamomi* (Table 1). All plants were grown in Mediterranean climate conditions in an experimental field located at the Institute for Sustainable Agriculture (IAS) (37.8ºN, 4.8ºW). At the end of their life cycles, seeds were collected, washed, lyophilized, and ground to fine powders using a Janke and Kunkel blender (Model A10 mill, IKA Labortechnik). GSL composition for each species and genotype was determined by High Performance Liquid Chromatography (HPLC) according to Font *et al*. (2005), and also described by Ríos *et al*. (2016a).

Oomycete material and *in vitro* experiments

All the experiments were carried out with *P. cinnamomi* strain PE90, isolated from *Quercus ilex* spp. *ballota*, and previously characterized as aggressive (Caetano *et al*., 2009). *In vitro* experiments were performed to test the ability of *Brassica* seedmeals to inhibit *P. cinnamomi* mycelial growth. Inoculum consisted of 6 mm diam. carrot agar (CA) plugs taken from the edges of 4-d-old *P. cinnamomi* colonies growing at 24ºC in the dark. Plugs were transferred to the centres of Petri dishes (9 cm diam.) containing fresh CA medium. Inoculated dishes were immediately inverted and placed as lids on the open tops of plastic beakers (9 cm internal upper diam.; 120 mm height; 0.4 L capacity), each containing lyophilized seed material plus 10 mL of deionized water. Four seedmeal doses were tested: 0 (experimental control), 0.2, 0.5 or 1.0 g of seed powder. Beakers were sealed with Parafilm® to avoid loss of volatiles. Four beakers (replicates) were prepared for each biofumigant and dose, and placed in a completely randomized experimental design. The experiment was repeated four times. The radial growth of colonies was measured daily for 4 d, which was the time it took for controls to completely colonize the dishes. At that time, dishes were removed from the beakers, covered with sterile Petri dish lids, and re-incubated

Table 1. Provenance of *Brassica* spp. and genotypes used in this study.

Brassica sp.	Genotype	Provided by
B. napus	Bn-Lewis	Dr. Delourne INRA, Rennes, France
	Bn-Salamander	
B. carinata	Bc-IAS-C1	Dr. de-Haro Plant Breeding Group IAS, Córdoba, Spain (Font *et al*., 2006)
	Bc-IAS-119	
B. juncea	Bj-Tezla	
	Bj-552	

for 1 week at 24° C in the dark to evaluate colony growth post-exposure to the volatiles.

Data of maximum radial growth were recorded after 3 d incubation. A two-way ANOVA was performed for maximum radial growth, with biofumigant genotype and dose as independent variables. Homoscedasticity was checked using Levene's test. When significance was obtained for $P < 0.05$, mean values were compared by Tukey´s HSD test at $\alpha = 0.05$ (Statistix software 9.0).

Soil experiments

One genotype each of *B. napus*, *B. carinata* or *B. juncea* was selected to be tested for effects on viability of resting spores (chlamydospores) of *P. cinnamomi* in soil. Natural soil was taken from an asymptomatic *dehesa* located in the north of Córdoba province (southern Spain). The soil was typical of *dehesa* systems in Córdoba: acidic with low fertility (Parras-Alcántara *et al*., 2014). The absence of the pathogen in ten soil samples was assessed following the method reported by Romero *et al*. (2007). No pathogen colonies were recovered from any sample.

The soil was air dried immediately after collection, sieved (2 mm mesh size), and artificially infested with a water suspension of *P. cinnamomi* chlamydospores prepared as described in Romero *et al*. (2007). Isolate PE90 of *P. cinnamomi* was plated in Petri dishes (9 cm diam.) containing 20 mL of carrot broth (20%) and incubated at 24°C in the dark. After 4 weeks of incubation, the liquid medium was aseptically filtered and the collected mycelium was washed with sterile deionized water. Washed mycelium was suspended in sterile water at a rate of three Petri dishes per 100 mL and placed in a blender (Pulsematic 16, Oster™) for 3 min at maximum speed (liquefy), to break up the mycelial aggregates and obtain free chlamydospores. Aliquots were taken from the homogenized suspension, and chlamydospores counted in a Neubauer counting chamber (0.1 μL). Chlamydospore concentration was adjusted to 1.5×10^4 mL^{-1}. The inoculum was carefully mixed with the soil to obtain a final concentration of 650 chlamydospores g^{-1} of dry soil. Lyophilized seed meal material (0.1, 0.5, or 1.0 g) was then placed at the bottom of 250 mL capacity plastic beakers. Beakers containing biofumigant-free soil were included as experimental controls. Infested soil (225 mL; 292.5 g) was poured into each beaker and the beaker was immediately covered, hand shaken, and mixed before adding water at field capacity (20% w/w water content). All beakers were incubated in a growth chamber in the dark, with the temperature at a 24°C (12 h) and 16°C (12 h) cycle. Incubation times were 1, 4 and 8 d. Four replicate beakers were prepared for each biofumigant species, dose and incubation time treatments, tested in a completely randomized experimental design. After each incubation period, soils were air dried for 4 d at room temperature. Ten grams of homogenized dry soil per beaker were suspended in 100 mL of 0.2% sterilized water-agar (Roko Industries), shaken and analysed following the method of Romero *et al*. (2007): 1 mL aliquots were taken from each soil-water-agar mix, and plated onto *Phytophthora* selective NARPH medium (Hüberli *et al*., 2000), using a sterile glass spreader to distribute the material over the agar surface. For each soil sample, a total of 20 Petri dishes were prepared. Dishes were incubated at 24°C for 24 h in the dark before washing the agar surface of each dish with sterile water to remove the soil-water-agar mix. Dishes were incubated again at 24°C for an additional 48 h in the dark. Growing colonies were identified as *P. cinnamomi* based on hyphal morphology observed under an inverted microscope (presence of rounded hyphal swellings in clusters together with chlamydospores), and were counted (Romero *et al*., 2007). Inoculum concentrations were expressed as colony forming units per g of dry soil (CFU g^{-1}). Data were transformed $[(\text{CFU g}^{-1}) + 0.5]^{1/2}$, and a three-way ANOVA was performed considering biofumigant, dose and incubation period as independent variables. Homoscedasticity was checked by Levene's test. When significance was obtained at $P < 0.05$, mean values were compared by the Tukey´s HSD test at $\alpha = 0.05$ (Statistix software 9.0).

Plant experiments

After sampling for chlamydospore viability, dried soils biofumigated with different doseages of the same seedmeals were mixed, and the same process was followed for unfumigated control soils. Homogenised soils (three soils treated with the three biofumigant species plus the untreated control soil) were distributed into 40 plastic pots per biofumigant (replicates, 75 mL of soil per pot).

Seeds of *Lupinus luteus* L., a species highly susceptible to *P. cinnamomi* (Serrano *et al*., 2010), were

germinated in damp chambers and when the radicles were approximately 3 cm long, seedlings were planted individually in the pots, making a total of 40 seedlings (replicates) per biofumigated or control soil. All pots were incubated at 24ºC (day) /16ºC (night) with constant irrigation, keeping the soil moisture close to 100% water holding capacity. After 1 month, symptoms of *P. cinnamomi* root disease were evaluated based on the percentage of root necrosis using a 0-4 scale; where 0 = 0% necrotic root, 1 = 1–33%, 2 = 34–66%, 3 = more than 67% necrotic root, and 4 = dead root (Serrano *et al*., 2010). Root symptom data were submitted to a one-way ANOVA with biofumigant as independent variable. Homoscedasticity was checked by Levene's test and mean values compared using the Tukey´s HSD test at $\alpha = 0.05$.

Ten plants were chosen at random from each biofumigant and control treatment, and six root segments per plant were plated on NARPH medium for re-isolation of the pathogen.

Results

GLS profiles

GSL profiles of the biofumigant seeds tested are shown in Table 2. Significant amounts of ten GSLs were identified and quantified: seven were aliphatic compounds (progoitrin, epiprogoitrin, sinigrin, gluconapoleiferin, glucoalyssin, gluconapin, and glucobrassicanapin), two were indolic compounds (glucobrassicin and 4-hydroxyglucobrassicin), and one was an aromatic compound (gluconasturtin) (Table 2). The GSL content of Bn-Lewis and Bn-Salamander seeds confirmed them, respectively, as double low and single low (high GSL) varieties. The content of all the aliphatic GSLs (especially progoitrin, R-2-hydroxy-3-butenyl glucosinolate) was less in Bn-Lewis compared with Bn-Salamander, with no major differences between the two genotypes in their contents of indolic or aromatic GSLs. All genotypes of *B. carinata* and *B. juncea* had high GSL contents with aliphatic GSLs accounting for >90% of the total; sinigrin (2-propenyl glucosinolate) was the most abundant GSL.

Inhibition of mycelial growth

Average values ± SE of maximum radial growth reached by *P. cinnamomi* colonies exposed to volatiles from biofumigants tested are presented in Table 3. ANOVA showed statistically significant differences among biofumigant dosages (DF = 3, F = 1169.23, $P<0.0001$), genotype (DF = 5, F = 898.66, $P<0.0001$) and the interaction dosage × genotype (DF = 15, F = 106.70, $P<0.0001$). At every dosage tested, genotypes of *B. carinata* and *B. juncea* reduced the mycelial growth of *P. cinnamomi* when compared with dose 0. The genotypes Bc-IAS-C1, Bc-IAS-119 and Bj-552 each gave total inhibition of *P. cinnamomi* mycelial growth, even at the lowest dosage tested. In contrast, colonies exposed to both genotypes of *B. napus* did not differ from control colony growth, even at the highest dosage tested (Table 3).

Table 2. Glucosinolate profiles and concentrations (μmol g^{-1} dry weight) in seeds of the selected *Brassica napus, B. carinata* and *B. juncea* genotypes.

***Brassica* spp.**	**Genotype**	**Total GSLs**	**Aliphatic**								**Indolic**	**Aromatic**	**Others**
			PRO[a]	**E-PRO[a]**	**SIN[a]**	**GNL[a]**	**GAL[a]**	**GNA[a]**	**GBN[a]**	**GBS[a]**	**4-OHGBS[a]**	**GST[a]**	
B. napus	Bn-Lewis	15.94	7.29	0.10	0.00	0.21	0.00	3.39	0.93	0.12	3.29	0.37	0.25
	Bn-Salamander	87.84	59.46	1.43	0.00	6.84	0.74	7.23	5.11	0.07	3.98	1.80	1.18
B. carinata	Bc-IAS-C1	86.01	1.47	0.00	79.36	0.00	0.00	0.66	0.00	0.45	2.86	0.46	0.75
	Bc-IAS-119	103.55	3.68	0.00	90.70	0.00	0.14	2.67	0.00	0.29	5.33	0.41	0.33
B. juncea	Bj-Tezla	87.33	0.53	0.00	79.16	0.00	0.00	4.16	0.00	0.05	2.14	0.53	0.86
	Bj-552	105.10	0.00	0.00	99.88	0.00	0.03	0.65	0.07	0.01	3.36	0.17	0.95

[a] Abbreviation of GSL trivial names: PRO, progoitrin; E-PRO, epiprogoitrin; SIN, sinigrin; GNL, gluconapoleiferin; GAL, glucoalyssin; GNA: gluconapin; GBN, glucobrassicanapin; GBS, glucobrassicin; 4-OHGBS, 4- hydroxyglucobrassicin; GST, gluconasturtin.

Table 3. Maximum radial growth (± SE) of *Phytophthora cinnamomi* colonies on CA medium after 3 d exposure to volatiles released by different *Brassica* spp. biofumigant seedmeals. Values accompanied by different letters differ significantly according with Tukey´s HSD test (P<0.05).

Brassica spp.	Genotype	Dose (g)			
		0.0 (control)	0.2	0.5	1.0
B. napus	Bn-Lewis	28.8 ± 0.6 a	28.3 ± 0.8 a	27.8 ± 1.0 a	28.3 ± 0.6 a
	Bn-Salamander	29.8 ± 0.3 a	27.0 ± 0.4 a	27.8 ± 0.5 a	27.5 ± 0.3 a
B. carinata	Bc-IAS-C1	29.5 ± 0.5 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	Bc-IAS-119	29.8 ± 0.6 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
B. juncea	Bj-Tezla	29.3 ± 0.8 a	9.3 ± 3.1 b	0.0 ± 0.0 c	0.0 ± 0.0 c
	Bj-552	28.8 ± 0.3 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c

Table 4. Average numbers (± SE) of viable *Phytophthora cinnamomi* chlamydospores (CFU g^{-1} of dry soil) after soil exposure to volatiles released by different *Brassica* biofumigants seeds at different doses and exposure times. Values with different letters in each column differ significantly according with Tukey´s HSD test (P<0.05).

Brassica spp.	Genotype	Dose (g)	Exposure time (d)		
			1	4	8
Control	-	0.0	104.7 ± 14.2 a	126.5 ± 13.0 a	106.6 ± 5.8 a
		0.1	100.7 ± 8.1 a	94.7 ± 17.0 a	90.1 ± 3.2 a
B. napus	Bn-Salamander	0.5	103.1 ± 4.2 a	94.7 ± 16.5 a	99.5 ± 10.1 a
		1.0	102.1 ± 4.5 a	88.1 ± 6.3 a	86.9 ± 31.5 a
		0.1	11.8 ± 1.9 b	7.9 ± 2.1 b	7.7 ± 0.5 b
B. carinata	Bc- IAS-119	0.5	13.2 ± 1.8 b	20.5 ± 4.8 b	6.4 ± 1.0 b
		1.0	14.6 ± 2.8 b	15.8 ± 5.4 b	5.0 ± 1.6 b
		0.1	12.1 ± 1.9 b	8.0 ± 1.4 b	8.5 ± 2.3 b
B. juncea	Bj-552	0.5	13.4 ± 2.3 b	5.7 ± 1.5 b	6.0 ± 1.4 b
		1.0	7.2 ± 1.8 b	20.7 ± 5.9 b	2.9 ± 0.7 b

When Petri dishes were re-incubated after exposure to the volatiles, colonies in those previously exposed to *B. carinata* or *B. juncea* remained unable to grow. In contrast, all cultures exposed to volatiles released by *B. napus* genotypes resumed growth and filled the plates after 1 week of incubation at 24ºC.

Chlamydospore viability

Bn-Salamander was the genotype of *B. napus* selected to test ability to inhibit chlamydospore viability in soil, as it was the genotype with the greatest GSL content. Genotypes Bc-IAS-119 and Bj-552 were selected since they gave 100% inhibition of mycelial growth *in vitro*, even at the lowest dosage tested. In addition, they were the genotypes with the highest GSL contents for, respectively, *B. carinata* and *B. juncea*.

Results obtained are presented in Table 4. ANOVA showed statistically significant differences in the viability of chlamydospores in soil, depending on the biofumigant seedmeal employed (DF = 2, F =

289.55, $P<0.0001$), but independent of dosage (DF = 2, F = 0.06, $P=0.9386$) or exposure time (DF = 2, F = 0.71, $P=0.4958$).

Chlamydospore viability in soils treated with seedmeal of Bn-Salamander did not differ from infested and unfumigated control soils. In contrast, genotypes Bc-IAS-119 and Bj-552 decreased chlamydospore viability, since the first day of exposure. No statically significant differences were observed between genotypes Bc-IAS-119 and Bj-552.

Effects on disease symptom development

After 1 month of incubation, *Lupinus* seedlings growing in control soils (infested and not biofumigated) developed foliar and root symptoms typical of the root rot caused by *P. cinnamomi*. Foliar symptoms included yellowing, foliar wilting, and defoliation. Root symptoms mostly included root necrosis. Figure 1 shows mean values recorded for root rot symptoms. ANOVA revealed statistically significant differences in root symptoms, depending on the biofumigant applied to the soil (DF = 3, F = 43.87, $P<0.0001$). As expected, root symptoms did not differ for *Lupinus* plants growing in soils treated with Bn-Salamander or growing in untreated control soil. However, roots symptoms were less when plants grew in soils biofumigated with Bc-IAS-119 or Bj-552 in comparison with plants in control soils. Bj-552 had the largest effect on decreasing root necrosis by *P. cinnamomi*. *Phytophthora cinnamomi* was always re-isolated from necrotic root segments from plants growing in every soil inoculated with this pathogen.

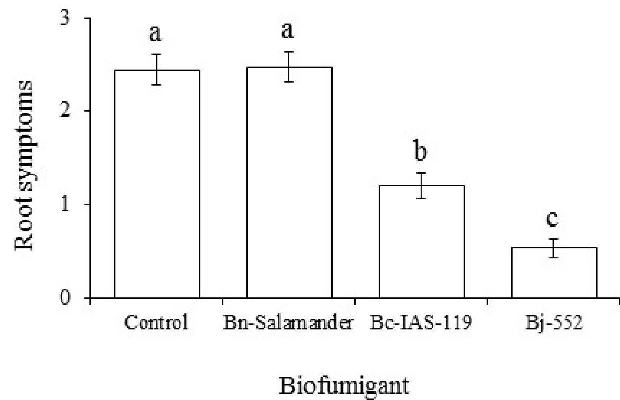

Figure 1. Average root symptom severity scores (and SE) for *Lupinus luteus* plants growing in soils infested with *P. cinnamomi* chlamydospores and biofumigated. Values with different letters differ significantly according with the Tukey´s HSD test ($P<0.05$).

Discussion

Brassica species contain GSLs in all their organs, but GSL concentration in seeds is especially high (Halkier and Gershenzon, 2006). However, breeding programmes have drastically reduced GSL levels in order to make *Brassica* genotypes more edible for livestock and humans (Tripathi and Mishra, 2007). Ríos *et al*. (2016a; 2016b) showed that sinigrin (2-propenyl glucosinolate) present in the green parts of some *Brassicaceae* genotypes had a high biofumigant potential against the oomycete *P. cinnamomi*. Green biofumigation using plants rich in sinigrin reduced the germination percentage of *P. cinnamomi* resting spores in soil. However, it should be noted that this green matter did not significantly reduce disease symptoms in highly susceptible "*dehesa*" hosts (Ríos *et al*. 2016a; 2016b). Even though commercial pellets based on *B. carinata* dry green matter have been reported to reduce *P. cinnamomi* inoculum density in experimental conditions, efficacy on live host roots was only demonstrated for seedlings of *Quercus cerris*, a host that is only very moderately susceptible to the pathogen (Morales-Rodríguez *et al*., 2016).

In the present study, we have demonstrated the ability of *Brassica* seedmeals rich in sinigrin to effectively reduce the viability of *P. cinnamomi* chlamydospore in soil. High efficacy was proven even at low dosages (0.1 g) and short exposure to volatiles (1 d), resulting in a 90% decrease in measured inoculum concentration in soil. This high efficacy may explain why increasing dosages or extending exposure time did not result in any additional effects on the viability of chlamydospores. As a result of the treatment, inoculum viability in soil was too low to result in root infections of *L. luteus* plants. Ríos *et al*. (2016a) reported inoculum loads of 28–69 CFU g^{-1} and 23–83 CFU g^{-1} were still detectable in soils after their biofumigation with green manures of, respectively, *B. juncea* Bj-552 or *B. carinata* Bc-IAS-119. Those inoculum concentrations are sufficient to induce root necrosis in lupins. Inoculum loads in the soil after biofumigation with *Brassica* seedmeals were approx., ten-fold less than those reported by Rios *et al*. (2016a). Inocula were estimated at 2-13 CFU g^{-1} for Bj-552 seedmeal treatments, and 5–20 CFU g^{-1} for Bc-IAS-119.

Serrano *et al*. (2015) reported a minimum of 61 chlamydospores g^{-1} is necessary to cause root disease on seedlings of the highly susceptible host *Q. suber* (Robin *et al*., 2001). Consequently, biofumigation with low doses of Bj-552 or Bc-IAS-119 seedmeals may protect cork oaks from root disease caused by *P. cinnamomi* infections.

This greater effectiveness of seedmeals in comparison with green matter appears to be directly related to their greater sinigrin concentration: four times for Bj-552 and six times for Bc-IAS-119 seedmeal in comparison with green matter (Ríos *et al*., 2016a). As previously reported (Ríos *et al*., 2016b), high sinigrin content is likely to be directly related to the biofumigant effectiveness of Brassicaceae plants against *P. cinnamomi*. Genotype Bj-552, with 100 *μmol* g^{-1} dry weight of sinigrin, was the most effective seedmeal for decreasing root necrosis induced by *P. cinnamomi* infections in lupin plants, followed by Bc-IAS-119, which contained 91 *μmol* sinigrin g^{-1} dry weight. However, the greater effectiveness achieved by Bj-552 in comparison with Bc-IAS-119 in reducing lupin root necrosis was not fully explained based on their sinigrin contents, and other factors depending on the species or genotype may be also involved.

We have shown here that *B. napus* Bn-Salamander seedmeal, rich in GSLs other than sinigrin, was ineffective for controlling *P. cinnamomi*, both in soil and *in planta*. This supports results previously reported by Ríos *et al*. (2016b) on the importance of sinigrin as an active biofumigant ingredient.

Phytophthora cinnamomi was always re-isolated from necrotic roots from inoculated soils, even when low levels of root necrosis were recorded. As Serrano *et al*. (2015) previously reported, very low concentrations of chlamydospores in soil can infect susceptible roots, even when the inoculum concentration is great not enough for disease development in the short term. Thus, although seedmeal biofumigation can effectively minimize levels of root disease caused by *P. cinnamomi* on highly susceptible hosts, it is not expected that it will eradicate inoculum. As stated by Dunstan *et al*. (2010), a site should be regarded "at risk" whenever the pathogen is detectable in the soil. Consequently, the risk for root disease development may remain in the long term if *P. cinnamomi* is still detectable in *dehesa* soils, even after biofumigation with effective seedmeals.

Our results indicate that the direct application to infested soil of sinigrin-rich *B. juncea* or *B. carinata* seedmeals is likely to be an effective way to control *Phytophthora cinnamomi*, and as such it should be incorporated in the integrated control of oak disease in *dehesa* systems. However, maximum production levels of *Brassica* spp. seed in *dehesa* were computed to be about 118 kg ha^{-1} (Fernández-Rebollo *et al*., unpublished data); this value is almost 20 times less than production levels recorded in different settings (Fereres *et al*., 1983). Hence, we infer that seedmeal to be used as a biofumigant in "*dehesas*" should not be locally produced, but should be supplied instead by production facilities in traditional agricultural lands.

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