Evaluating biofumigant crops as a pre-plant treatment for avocado orchard establishment in sites with high Phytophthora root rot pressure

E.K. Lancaster[1*], P. de Souza[1], D. P. Le[1], J. Duff[2], and E.K. Dann[1]

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia.

[2] Queensland Department of Agriculture and Fisheries, Australia.

* Corresponding author: e.lancaster@uq.edu.au

Summary

Brassica biofumigant crops are frequently grown and incorporated into fields prior to planting vegetable crops to reduce populations of nematodes and soilborne fungi. This approach was evaluated for application in avocado replant land infested with *Phytophthora cinnamomi* (Pc). *In vitro* Petri dish assays demonstrated that growth of Pc was inhibited by amending agar with dried leaf material of several brassica species, with Caliente (*B. juncea*) and BQ Mulch (a mix of *B. napus* and *B. campestris*) being most effective. However, these results were not observed during further glasshouse testing. The efficacy of these pre-plant biofumigants is currently being tested in a field trial on a commercial avocado orchard infested with Pc.

Introduction

Since the withdrawal of methyl bromide and many other environmentally damaging chemicals in recent years, brassica biofumigants have emerged as a viable option to manage soilborne pests and pathogens in many commercially important crops (Matthiessen & Kirkegaard 2006). Brassica species contain high levels of glucosinolate compounds in their above- and below-ground tissues, particularly at the flowering stage (Morales-Rodríguez et al. 2012). When these tissues are ruptured, glucosinolates come into contact with the enzyme myrosinase and are hydrolysed (when in the presence of free water), causing the release of volatile isothiocyanate compounds (Morales-Rodríguez et al. 2012).

Isothiocyanates are able to inhibit the growth of many populations of bacteria and fungi, which can substantially reduce pathogen load in the field (Wang et al. 2014; Hu et al. 2015). However, the inhibitory effects on each pathogen vary depending on the types of isothiocyanates produced, which is determined by the structure of the glucosinolate compounds present in the brassica species (Hu et al. 2015). Many commercial biofumigant mixes are now available and these may consist of a single species, such as *Brassica juncea* (“Caliente”, “Mustclean” and “Nemfix”), *Eruca sativa* (“Nemat”), *Raphanus sativus* (Tillage radish”), or a mix of species, such as *B. napus* with *B. campestris* (“BQ Mulch”) and *R. sativus* with *Sinapsis alba* (“Biofum”) that produce different glucosinolates.

Australian avocado orchards are challenged by a variety of fungal pathogens that can substantially damage the root systems of infected trees, causing a decline in tree health and fruit quality. *Phytophthora cinnamomi* (Pc) in particular has caused widespread damage in commercial orchards, and growers typically rely on the use of phosphonates to maintain production (Pegg et al. 1990; Drenth & Guest 2004). Other studies have identified numerous nectriaceous fungi, such as *Calonectria ilicicola* (Ci) and *Dactylonectria macrodidyma* (Dm), which are also pathogens of avocado (Dann et al. 2012; Parkinson et al. 2017). There is some evidence to suggest that brassica biofumigant crops, particularly those rich in sinigrin, may be able to reduce the inoculum load of Pc (Morales-Rodríguez et al. 2016; Rios et al. 2016a & b). This could be a beneficial strategy for Australian avocado growers in areas with high Pc pressure to reduce the inoculum load and improve the survival of their trees. The aim of this study was to examine the biofumigant activity of several commercial brassica mixes and evaluate their viability as a pre-plant disinfestation strategy in Australian avocado orchards with high Pc inoculum. The biofumigant activity on two other fungal pathogens of avocado, Ci and Dm, was also assessed.

Materials and methods

Petri dish assays with powdered brassicas were used to test the biofumigant activity of Biofum, BQ Mulch, Caliente, Mustclean, Nemat and Tillage against Ci, Dm and Pc *in vitro*. The brassica treatments were prepared by harvesting plants (all above and below-ground tissue) that were grown to the full-flowering stage, then oven-dried at 70°C and ground to a fine powder. Molten agar was amended with 0.5, 1.0 or 2.0 g of powdered brassica (equivalent to 50, 100 and 200 t/Ha fresh respectively), then poured into plates. Once set, a plug of Ci, Dm or Pc was placed in the centre of the plate and colony diameter was measured. The activity of only the volatile compounds was assessed in a separate experiment. Powdered brassica (0.5 g, 1.0 g or 2.0 g, prepared as described previously) was placed in the bottom of a Petri dish, and then water added to activate. Another Petri dish containing agar and an inoculum plug was inverted and placed over the biofumigant. The Petri dish halves were taped together, ensuring that the brassica powder was not in direct contact with the fungus, but allowed dissipation of the antifungal gas. There were three replicate plates per treatment (or controls) in each experiment.

The biofumigant activity of Caliente, Nemat and composted chicken manure were further tested against Dm and Pc in two glasshouse trials using inoculated avocado seedlings planted in potting mix amended with the biofumigants. The experiment was established in a completely randomised design with 8-10 seedlings per treatment, as listed in Tables I and II. The avocado seedlings were transplanted into pots containing soil unamended (control) or amended with the brassica biofumigant (7 g DW) or composted chicken manure (20 g; sourced from a local avocado grower) and inoculated at the same time. All pots were watered and sealed with a plastic bag for two days to retain any volatile compounds produced. Plant heights were measured weekly until the trial was terminated five weeks after treatment application and inoculation. The roots of each plant were carefully washed and then evaluated for root necrosis (%). Fresh and dry weights of the root systems and above-ground tissues were also recorded. The entire experiment was repeated, terminated after six weeks.

The efficacy of BQ Mulch and Caliente as pre-plant biofumigants has been tested in a field trial on a commercial avocado orchard. A site with mature avocado trees that were in severe decline from Pc was selected. The trees were removed, and experimental plots (40 m^2) were sown with one of the two brassica biofumigants or left fallow (untreated control). When the brassica crops had grown to approximately 25% flowering, the plots were mown and the plant material was incorporated into the top 10 cm of soil with a rotary hoe. Chicken manure was spread and incorporated in other plots as a 4^{th} treatment. A split-plot design was used to evaluate the efficacy of covering the plots with black plastic (covered versus uncovered) for two weeks post-incorporation to increase the amount of gas retained in the soil. Replanting with Hass on Reed nursery stock occurred at four weeks post-incorporation, and then monthly tree health assessments (1-6 scale) were undertaken.

Results and discussion

In vitro studies investigating the biofumigant activity of the brassica plant tissue on the growth of Ci, Dm and Pc were promising, with a distinct inhibition of fungal growth in plates treated with brassica biofumigants (Figure 1). The growth of Pc was inhibited entirely in plates amended with BQ Mulch, regardless of concentration, and plates amended with 1.0 and 2.0 g of Caliente. There was also an apparent inhibition by volatile compounds emanating from moistened brassica tissue, demonstrating the "biofumigation" effect (Figure 2).

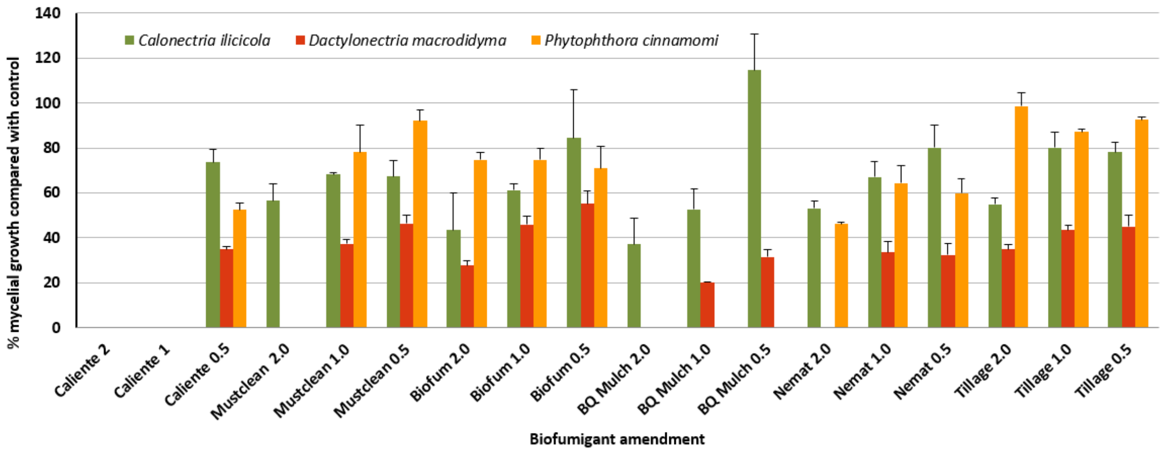

Figure 1. The effect of agar media amendments with powdered brassica biofumigants on the growth of three soilborne pathogens of avocado *in vitro* (*n*=3 replicate Petri dishes per treatment; bars represent SEM).

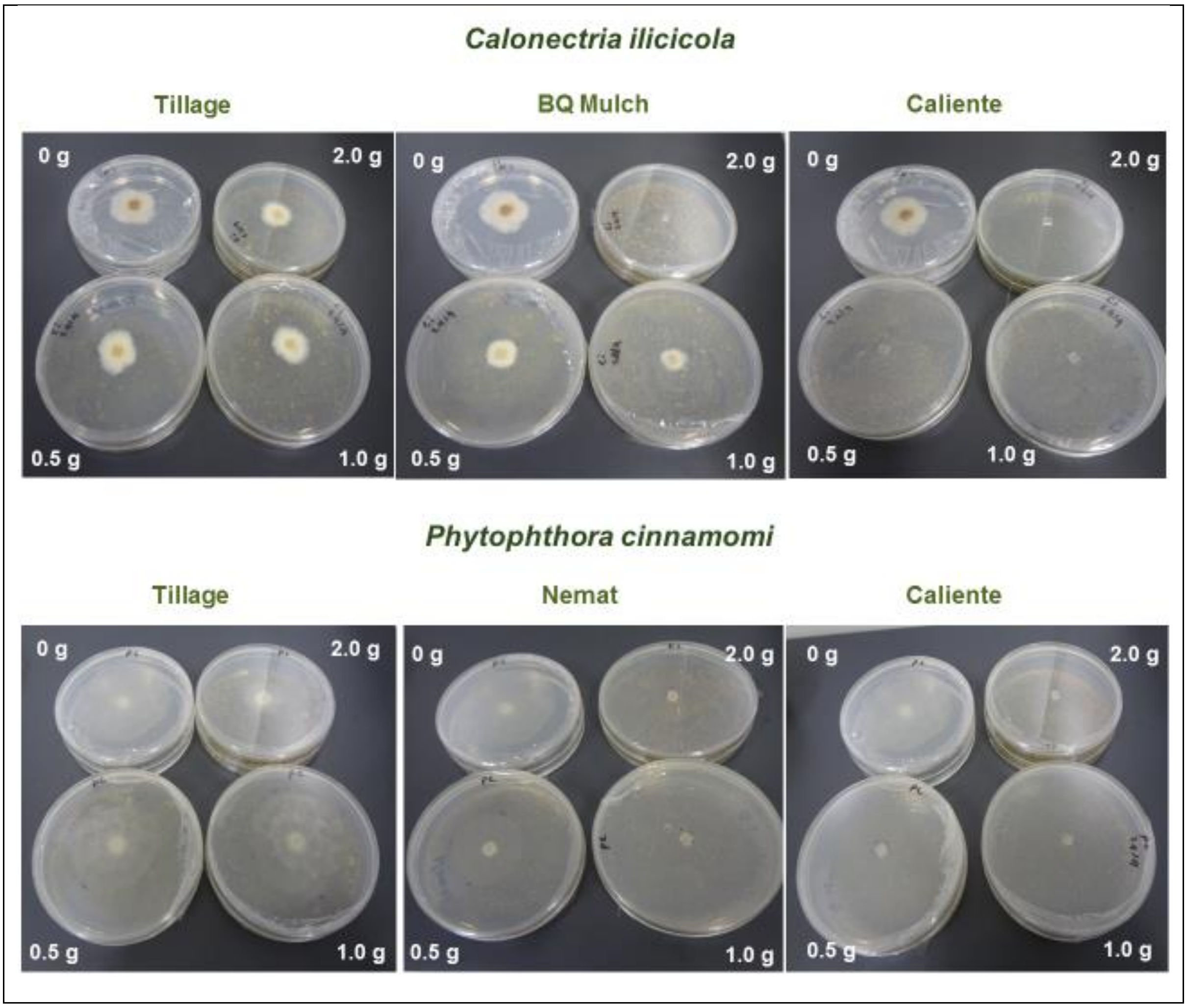

Figure 2. The effect of the brassica biofumigant volatile compounds of Tillage, Nemat and Caliente on the growth of Ci and Pc *in vitro*.

The glasshouse experiments examining the effect of brassica biofumigant amended potting mix on the health of inoculated avocado seedlings were less conclusive. In the first glasshouse experiment, amendment of the potting mix with Caliente reduced root necrosis in avocado seedlings by nearly 40% five weeks post-inoculation with Pc, compared to the inoculated control that received no soil amendment (Table 1). However, Caliente did not reduce root necrosis caused by Dm. In the second glasshouse experiment, root necrosis after inoculation with Dm was reduced by Caliente and Nemat amendment, but not significantly (Table 2). There was no effect of Caliente improving root health after inoculation with Pc.

Table 1. The effect of two biofumigants, Nemat and Caliente, and composted chicken manure on growth, biomass and root necrosis of avocado seedling cv. Hass, five weeks after inoculation with soilborne pathogens Pc or Dm, Experiment 1

Treatment	n	Change in height (cm)	Leaves+ stems FW (g)	Roots FW (g)	Leaves+ stems DW (g)	Roots DW (g)	% necrotic roots	
No treatment, no pathogen	8	14.8	26.6	16.6	6.69	1.54	18.8	e
No treatment, Pc	8	10.8	37.3	19.9	11.3	2.44	72.5	a
No treatment, Dm	8	18.8	47.9	22.7	12.0	2.01	45.0	bcd
Nemat, no pathogen	8	18.9	30.3	17.1	8.04	1.71	30.6	de
Nemat, Pc	8	16.2	35.8	21.6	9.35	2.11	62.5	ab
Nemat, Dm	8	19.1	39.4	21.4	9.93	2.38	45.0	bcd
Caliente, no pathogen	8	12.5	32.4	17.5	9.36	2.34	35.6	cde
Caliente, Pc	8	14.8	36.4	17.3	10.8	2.31	45.0	bcd
Caliente, Dm	8	13.8	29.7	14.5	7.12	1.53	52.5	abc
Chicken manure, no pathogen	8	14.4	39.2	24.2	10.5	2.62	16.3	e
Chicken manure, Pc	8	11.6	34.7	16.8	9.74	1.85	60.0	ab
Chicken manure, Dm	8	19.2	42.9	17.1	10.9	1.81	63.8	ab
P		**0.695**	**0.061**	**0.815**	**0.161**	**0.920**	**<0.001**	

For % necrotic roots means followed by the same letter(s) were not significantly different at *p*=<0.05. For all other parameters, there were no significant differences among treatments, as indicated by *p*=>0.05.

Table 2. The effect of Caliente, Nemat and composted chicken manure on the growth, biomass and root necrosis of avocado seedling (cv. Hass), six weeks after inoculation with the soilborne pathogens Pc or Dm, Experiment 2

Treatment	n	Change in height (cm)		Leaves+ stems FW (g)		Roots FW (g)		Leaves+ stems DW (g)		Roots DW (g)		% necrotic roots	
No treatment, no pathogen	10	3.5	a	25.3	ab	13.4	ab	6.80	a	1.88	b	9.5	e
No treatment, Pc	10	0.7	d	14.8	fg	5.05	de	4.30	cd	0.79	d	70.1	b
No treatment, Dm	10	2.9	ab	15.5	fg	4.04	e	4.01	cd	0.88	d	27.5	cd
Nemat, no pathogen	10	2.4	abc	21.3	bcde	12.1	abc	5.63	ab	1.75	b	13.3	e
Nemat, Pc	9	1.8	bcd	18.0	defg	10.7	bc	5.15	bc	1.53	bc	76.2	ab
Nemat, Dm	10	3.3	a	24.2	abc	9.40	c	5.97	ab	1.94	b	19.1	de
Caliente, no pathogen	10	3.1	ab	17.1	efg	9.93	bc	4.74	bcd	1.59	b	15.8	de
Caliente, Pc	9	1.2	cd	18.5	def	4.41	e	5.59	ab	0.95	cd	67.0	b
Caliente, Dm	10	3.2	a	21.6	bcd	10.2	bc	5.84	ab	1.77	b	20.5	de
Chicken manure, no pathogen	10	2.7	ab	20.3	cde	8.50	cd	4.96	bcd	1.74	b	15.3	e
Chicken manure, Pc	9	0.7	d	13.7	g	2.64	e	3.79	d	0.67	d	86.2	a
Chicken manure, Dm	10	3.3	a	26.4	a	14.7	a	6.86	a	2.67	a	35.2	c

Within each column, means followed by the same letter were not significantly different at *p*=<0.05.

The field trial evaluating the biofumigant activity of BQ Mulch, Caliente and chicken manure on a commercial avocado orchard, is still in progress. At five months post-planting, differences between the bio-fumigant treatments were not significant (p = 0.402), but trees planted in the plots covered with black plastic were significantly (p = <0.001) healthier than those in the uncovered plots (Figure 3). Soil samples collected immediately prior to replanting showed covered plots had significantly higher levels (ppm) of many nutrients including nitrate, ammonium, manganese, sulphur, chloride, magnesium and potassium.

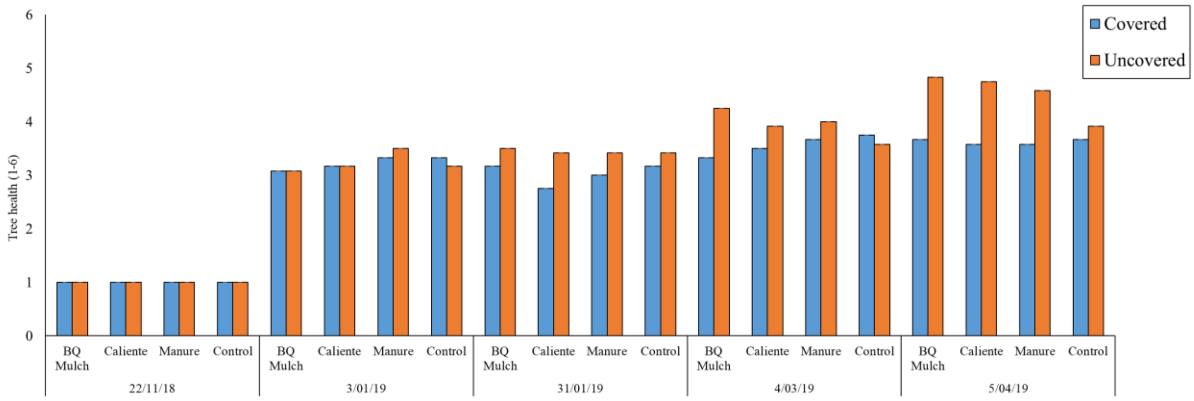

Figure 3. Tree health scores (means) of trees planted in covered and uncovered experimental plots treated with BQ Mulch, Caliente, chicken manure or the untreated control. Plants were assessed on a 1-6 scale (healthy =1, dead = 6) at each monthly assessment.

The results of these studies indicate that the incorporation of brassica biofumigant species as a pre-plant disinfestation strategy may be beneficial in Australian avocado orchards. *In vitro* testing of several commercial brassica biofumigant mixes have shown promising results against Pc, but this was not observed *in planta* when further testing was carried out in the glasshouse. However, although not statistically significant, higher root necrosis (%) was reported for uninoculated plants in the soil with biofumigant amendments, compared to the unamended control. This indicates that fresh biofumigant is also phytotoxic to avocado roots, and future experiments should ensure a period of at least 2 weeks interval between application of biofumigant treatment and planting with avocado. At the time of writing, the field trial testing commercial brassica mixes in a commercial orchard is still underway. Although differences between biofumigant or chicken manure treatments are not significant to date, tree health has been significantly better in plots covered by black plastic post-incorporation. Further research is needed to test the efficacy of the commercial brassica biofumigant mixes or pelletised products in avocado orchards with differing environmental conditions and soil types. Additional studies are also needed to examine the effect of using black plastic as a pre-plant strategy in sites known to be infested with Pc or other soilborne pathogens, and the cost-benefit ratio to growers.

Acknowledgements

This project has been funded by Hort Innovation, using the avocado industry research and development levies and contributions from the Australian Government. This project is jointly supported by the Department of Agriculture and Fisheries (DAF) and The University of Queensland (UQ). The authors would like to sincerely thank Anderson Horticulture Pty Ltd for the continued industry support.

References

Dann, E.K., Cooke, A.W., Forsberg, L.I., Pegg, K.G., Tan, Y.P. and Shivas, R.G., 2012. Pathogenicity studies in avocado with three nectriaceous fungi, Calonectria ilicicola, Gliocladiopsis sp. and Ilyonectria liriodendri. *Plant Pathology*, 61(5), pp.896-902.

Drenth, A. and Guest, D.I., 2004. 7.1 Principles of Phytophthora Disease Management. *Diversity and management of phytophthora in Southeast Asia*, pp.154-160.

Hu, P., Hollister, E.B., Somenahally, A.C., Hons, F.M. and Gentry, T.J., 2015. Soil bacterial and fungal communities respond differently to various isothiocyanates added for biofumigation. *Frontiers in Microbiology*, 5, p.729.

Matthiessen, J.N. and Kirkegaard, J.A., 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical reviews in plant sciences*, *25*(3), pp.235-265.

Morales‐ Rodríguez, C., Picón‐ Toro, J., Palo, C., Palo, E.J., García, Á. and Rodríguez‐ Molina, C., 2012. In vitro inhibition of mycelial growth of Phytophthora nicotianae Breda de Haan from different hosts by Brassicaceae species. Effect of the developmental stage of the biofumigant plants. *Pest Management Science*, 68(9), pp.1317-1322.

Morales-Rodríguez, C., Vettraino, A.M. and Vannini, A., 2016. Efficacy of biofumigation with Brassica carinata commercial pellets (BioFence) to control vegetative and reproductive structures of Phytophthora cinnamomi. *Plant Disease*, 100(2), pp.324-330.

Parkinson, L.E., Shivas, R.G. and Dann, E.K., 2017. Pathogenicity of nectriaceous fungi on avocado in Australia. *Phytopathology*, 107(12), pp.1479-1485.

Pegg, K.G., Whiley, A.W. and Hargreaves, P.A., 1990. Phosphonic (phosphorous) acid treatments control Phytophthora diseases in avocado and pineapple. *Australasian Plant Pathology*, 19(4), pp.122-124.

Ríos, P., Obregón, S., de Haro, A., Fernández- Rebollo, P., Serrano, M.S. and Sánchez, M.E., 2016a. Effect of Brassica biofumigant amendments on different stages of the life cycle of Phytophthora cinnamomi. *Journal of Phytopathology*, 164(9), pp.582-594.

Ríos, P., Obregón, S., González, M., de Haro, A. and Sánchez, M.E., 2016b. Screening brassicaceous plants as biofumigants for management of Phytophthora cinnamomi oak disease. *Forest Pathology*, 46(6), pp.652-659.

Wang, Q., Ma, Y., Yang, H. and Chang, Z., 2014. Effect of biofumigation and chemical fumigation on soil microbial community structure and control of pepper Phytophthora blight. *World Journal of Microbiology and Biotechnology*, 30(2), pp.507-518.