Australasian Plant Pathol. (2012) 41:347–349
DOI 10.1007/s13313-012-0142-6

Brassica green manure rotation crops reduce potato stem rot caused by *Sclerotinia sclerotium*

Mohammad Reza Ojaghian · Zhou-qi Cui · Guan-Lin Xie · Bin Li · Jingze Zhang

Received: 3 July 2011 / Accepted: 3 April 2012 / Published online: 21 April 2012

Abstract This study was conducted to evaluate the potential of biofumigation in three Brassica crops including *Brassica napus*, *Brassica juncea* and *Brassica campestris* against potato stem rot caused by *Sclerotinia sclerotiorum* in field tests. Results from field trials carried out in three naturally infected potato fields during three cropping seasons of 2008–2010 showed that the Brassica crops used as green manure cover crops were able to significantly reduce disease incidence and mean percentage of dead plants (as a proportion of infected plants). Although results varied somewhat by field site and year, *B. juncea* generally provided the highest level of control, averaging greater than 55.6 % reduction in disease incidence over all fields and years, compared to average disease reductions of 31.6 and 45.8 % for the *B. napus* and *B. campestris* crop treatments, respectively. Furthermore reduction of dead plants averaged 61.6, 39.2 and 32.1 % for *B. juncea*, *B. napus*, and *B. campestris*, respectively. In this study, Brassica crops showed various significant inhibitory effects in different fields and years indicating that disease development is affected by other factors including environmental conditions.

Keywords Agria · Biofumigation · Brassicaceae · Field tests

Potato (*Solanum tuberosum* L.) stem rot is a disease which causes considerable yield loss in numerous fields of Hamadan province, Iran (Ojaghian 2009). The causal agent is *Sclerotinia sclerotiorum* (Lib.) de Bary, an ascomycetous and soilborne fungus. The symptoms are mostly observed on the lower parts of stems at final stages of growing season; therefore this disease is not usually recognized and regarded by potato growers. Because most of tuber formation is carried out during last weeks of cropping season, the control of this pathogen may result in increased crop production.

Biofumigation, defined as the suppression of non-desirable soil-borne organisms by biocidal compounds released into the soil during the decomposition of plant material has been reported to be beneficial for suppressing soilborne pathogens etc. (Matthiessen and Kirkegaard 2006). Plants in the *Brassicaceae* contain glucosinolates (GSLs), and their hydrolysis products (isothiocyanates (ITCs) involve in suppression of soilborne organisms. The GSLs are not biologically active, they must be enzymatically hydrolyzed by the enzyme myrosinase to ITCs nitriles, thiocyanates, and oxazolidinethiones that are capable of suppressing soil pathogens (Bones and Rossiter 1996).

The objective of this study was to evaluate the potential of biofumigation in three Brassica crops as green manure against potato stem rot in field trials. The biocontrol efficacy of volatile materials produced from the Brassica crops had previously been tested against five isolates of the pathogen in preliminary in vitro experiments. They were able to reduce radial growth and sclerotia formation of all isolates ranging from 27–92 % and 35–93 %, respectively (Ojaghian et al. 2012).

During the cropping seasons of 2008, 2009 and 2010, nine field tests were established in three potato fields naturally infested with high levels of the pathogen (Field 1, Field 2 and Field 3) located in Bahar, Hamadan. Field experiments were arranged in a randomized complete block design with three treatments including canola, Indian mustard and Ida gold field mustard and each treatment was replicated four, three and four times in 2008, 2009 and 2010, respectively. Individual plot sizes were 4×5, 4×5 and 3.5×5.5 m

---

M. R. Ojaghian · Z.-q. Cui · G.-L. Xie (✉) · B. Li · J. Zhang
State Key Laboratory of Rice Biology, Institute of Biotechnology,
Zhejiang University,
Hangzhou 310058, People's Republic China
e-mail: glxie@zju.edu.cn

Table 1 Inhibitory efficacy of three Brassica crops as green manure against incidence of *S. sclerotiorum* in potato fields of Hamadan, Iran during growing seasons of 2008–2010

Brasica spp.	Disease incidence (%)*								
	Field 1			Field 2			Field 3		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
Control	$69^{a}\pm24.4$	$59^{a}\pm31.2$	$75^{a}\pm29.8$	$78^{a}\pm14.2$	$51^{a}\pm19.5$	$37^{a}\pm19.6$	$56^{a}\pm25.4$	$45^{a}\pm21.1$	$33^{a}\pm14.1$
B. napus	$45^{b}\pm15.3$	$36^{b}\pm15.5$	$65^{b}\pm29.9$	$25^{c}\pm11.3$	$25^{b}\pm12.4$	$35^{a}\pm14.5$	$45^{b}\pm23.3$	$38^{b}\pm17.2$	$21^{b}\pm5.7$
B. juncea	$25^{c}\pm11.1$	$29^{c}\pm10.3$	$44^{cd}\pm17.8$	$21^{c}\pm9.1$	$17^{c}\pm6.5$	$33^{a}\pm13.2$	$24^{c}\pm8.9$	$16^{c}\pm5.1$	$10^{c}\pm4.6$
B. campestris	$49^{b}\pm19.9$	$33^{bc}\pm16.2$	$52^{c}\pm22.8$	$35^{b}\pm8.7$	$16^{c}\pm6.9$	$19^{b}\pm8.1$	$41^{b}\pm19.6$	$18^{c}\pm6.8$	$18^{b}\pm5.7$

* Values in the table indicate means ± standard error. Each treatment was replicated four, three and four times in 2008, 2009 and 2010, respectively. Within columns, means followed by the same letters are not different significantly ($P>0.05$) according to Fisher's test

in Field 1, Field 2 and Field 3, respectively. In addition, a 50-cm-wide border was maintained for each replication, and 2–3 m wide borders were maintained unplanted between blocks. All fields had already been under commercial production of potato for 5–6 years. As soon as the fields were harvested and waste tubers were removed, the soil was cultivated using a chisel plough. The fine seed bed needed for the small Brassica seeds was prepared with a power harrow working to about 8–11 cm. The planting date of Brassica crops was 14, 18 and 23 August in 2008, 2009 and 2010, respectively. Canola (*Brassica napus*), Indian mustard (*Brassica juncea*) and Ida gold field mustard (*Brassica campestris*) were grown in the test areas for 90–100 days when first flowers started to open. Control plots did not have any Brassica crop. Brassica plants were then mulched using a 4.5 m wide 4-row profiled pulverizer normally used to destroy potato stems in preparation for harvest. At this time, soil moisture was in the level that after squeezing the soil sample firmly in the hand it would form an irregularly shaped ball but fall apart when bounced in hand.

Immediately after pulverizing, the crushed tissues were incorporated into the soil using a moldboard plough. After 1 month, plots were disc ploughed three times at approximately two-week intervals. A deeper cultivation was then carried out with a chisel plough. No fertilizer was applied in 2008, whereas nitrogen fertilizer was spread in 2009 and 2010 at a rate of 50 and 60 kg/ha, respectively.

Potato tubers (cv. Agria) were planted at 4.8 t/ha at a row spacing of 40 cm in 25 May, 20 May and 10 June in 2008, 2009 and 2010, respectively. In 3 years, the herbicide Treflan 480EC containing 480 g/L of trifluralin (DowElanco, Calgary, Alberta, Canada) was incorporated into soil before sowing at a rate of 2 L/ha. The plots were irrigated as needed using a center pivot system, and plants were rated for incidence of stem rot by visual observation of symptoms (Purdy 1979) during harvesting (late September). Disease incidence (DI) was defined as the percentage of plants per plot infected by *S. sclerotiorum*. In addition, the mean percentage of dead plants (as a proportion of infected plants) per plot was determined.

The effects of different treatments in each experiment were determined using analysis of variance (ANOVA) with the SAS software (SAS 8.2, 1999–2001; SAS Institute Inc., Cary, NC) for field experiments. Means of treatments were separated using Fishers' LSD test.

The results of field experiments conducted at three different sites over three consecutive years demonstrated that,

Table 2 The effect of three Brassica crops as green manure on percentage of Sclerotinia-caused dead plants (as a proportion of infected plants) in three naturally infected potato fields of Hamadan, Iran during cropping seasons of 2008–2010

Brasica spp.	Dead plants (%)*								
	Field 1			Field 2			Field 3		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
Control	$34^{a}\pm24.4$	$25^{a}\pm31.2$	$31^{a}\pm29.8$	$45^{a}\pm14.2$	$26^{a}\pm19.5$	$19^{a}\pm19.6$	$29^{a}\pm25.4$	$31^{a}\pm21.1$	$16^{a}\pm14.1$
B. napus	$23^{b}\pm15.3$	$18^{b}\pm15.5$	$25^{b}\pm29.9$	$25^{c}\pm11.3$	$24^{a}\pm12.4$	$16^{a}\pm14.5$	$17^{ab}\pm23.3$	$18^{b}\pm17.2$	$4^{b}\pm5.7$
B. juncea	$21^{b}\pm11.1$	$6^{c}\pm10.3$	$8^{cd}\pm17.8$	$21^{c}\pm9.1$	$13^{b}\pm6.5$	$5^{c}\pm13.2$	$12^{b}\pm8.9$	$11^{bc}\pm5.1$	$6^{b}\pm4.6$
B. campestris	$19^{b}\pm19.9$	$15^{b}\pm16.2$	$13^{c}\pm22.8$	$36^{b}\pm8.7$	$21^{a}\pm6.9$	$11^{b}\pm8.1$	$26^{a}\pm19.6$	$15^{b}\pm6.8$	$18^{a}\pm5.7$

* Values in the table indicate means ± standard error. Each treatment was replicated four, three and four times in 2008, 2009 and 2010, respectively. Within columns, means followed by the same letters do not differ significantly ($P<0.05$) according to Fisher's test

overall, all three Brassica residue treatments, with the exception of field trial 2 in 2010, significantly reduced ($P>0.05$) the incidence of potato stem rot (DI) relative to the untreated control (Table 1), and in most cases also reduced ($P<0.05$) the number of dead plants (Table 2). Although results varied somewhat by field site and year, *B. juncea* (Indian mustard) generally provided the highest level of control, averaging greater than 55.6 % reduction in DI over all fields and years, compared to average disease reductions of 31.6 and 45.8 % for the *B. napus* (canola) and *B. campestris* (Ida gold field mustard) crop treatments, respectively. Furthermore reduction of dead plants averaged 61.6, 39.2 and 32.1 % for Indian mustard, canola, and field mustard, respectively.

This study has demonstrated that Brassica crops, and particularly *B. juncea*, can be used to consistently and effectively reduce disease problems caused by potato stem rot. The Brassica crops were generally effective over multiple years and in multiple different fields, suggesting that their use could greatly diminish losses due to potato stem rot in this region. The percentage of dead plants, as a more developed form of diseased plants, was also reduced in most trials.

Grown as green manure, Indian mustard showed the best results of all tested Brassica crops in most field trials, and it may be due to production of higher amount or more effective inhibitory compounds (Charron and Sams 1999; Smolinska and Horbowicz 1999).

In most field tests, Brassica crops were not able to decrease disease incidence and dead plants at statistically similar level. In field 2(2010), for example, *B. campestris* was statistically the most effective treatment against disease incidence but *B. juncea* showed the most inhibitory effect against dead plants. Brassica crops, on the other hand, showed significantly different inhibitory effect in different fields. These observations show that disease development is affected by other factors including environmental conditions. Larkin and Griffin (2007) have shown that the reduction of *Rhizoctonia solani* in potato fields subject to Brassica crops rotation is not always closely associated with biofumigation potential.

In field 2, the percentage of DI in controls was reduced from 78 to 37 in 2008 and 2010, respectively. Disease incidence was also decreased in field 3 from 56 to 45 and 33 in 2008, 2009 and 2010, respectively. It shows that the *Brassica* rotation may need to go through some successive years to attain optimum results. In some geographical regions, pulverized crops may carry huge problematic moisture into soil giving rise the occurrence of other diseases. In this study, although Brassica crops were incorporated into soil when they were 100 day old, it is essential to determine the suitable pulverizing time of Brassica crops depending on mean temperature and air humidity. Consequently it may be necessary to prevent crops from developing too much biomass or it may be required to give more time between ploughing and planting potatoes.

Acknowledgements This project was supported by the Special Fund for Agro-scientific Research in the Public Interest (201003029, 201003066). The authors wish to acknowledge their gratitude to Abdulwareth Almoneafy, Heng Jiang, Wen-Xiao Tian, Guo-Qing Zhang and He Liu.

References

Bones A, Rossiter JT (1996) The myrosinase-glucosinolate system, its organization and biochemistry. Physiol Plantarum 97:194–208

Charron CS, Sams CE (1999) Inhibition of Pythium ultimum and Rhizoctonia solani by shredded leaves of Brassica species. J Am Soc Hort Sci 124:462–467

Larkin RP, Griffin TS (2007) Control of soilborne potato diseases using *Brassica* green manure. Crop Prot 26:1067–1077

Matthiessen JN, Kirkegaard JA (2006) Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Crit Rev Plant Sci 25:235–265

Ojaghian MR, Jiang H, Xie GL, Cui Z-Q, Zhang J, Li B (2012) In vitro biofumigation of Brassica tissues against potato stem rot caused by *Sclerotinia sclerotiorum.* Iran J Agri Sci (in press)

Ojaghian MR (2009) First report of *Sclerotinia sclerotiorum* on potato plants in Iran. Australasian Plant Dis Notes 41:39–41

Purdy LH (1979) Sclerotinia sclerotiorum: history, diseases and symptomatology, host range, geographic distribution and impact. Phytopathol 69:875–880

Smolinska U, Horbowicz M (1999) Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. J Phytopathol 147:119–124