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Short communication

Use of formulated *Trichoderma* sp. Tri-1 in combination with reduced rates of chemical pesticide for control of *Sclerotinia sclerotiorium* on oilseed rape

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ABSTRACT

Sustainable strategies for control of *Sclerotinia sclerotiorum* on oilseed rape are needed. Here we tested combinations of *Trichoderma* sp. Tri-1, formulated with oilseed rape seedcake and straw, with reduced application rates of the recommended chemical pesticide carbendazim for control of this pathogen on oilseed rape. The treatment containing the recommended rate of carbendazim provided the greatest reduction in disease when compared with treatments containing individual applications of lower rates of this pesticide or the formulated Tri-1 treatment in all field experiments. Treatments containing formulated Tri-1 combined with carbendazim applied at 50% or 75% the recommended rate reduced incidence of disease to levels statistically similar to the treatment containing carbendazim applied at the recommended rate in two field experiments conducted at the Wuxue County field site where a rice-oilseed rape rotation was used. The treatment containing formulated Tri-1 combined with carbendazim applied at 75% the recommended rate reduced incidence of disease to levels statistically similar to the treatment containing carbendazim applied at the recommended rate in two field experiments conducted at the Wuhan field site where a soybean-oilseed rape rotation was used. Experiments reported here using two oilseed rape production rotations indicate that integration of a biologically based control tactic, such as formulated Tri-1, into a disease management strategy can increase oilseed rape production sustainability through reduction in the use of chemical pesticides.

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Sclerotinia sclerotiorum (Lib.) de Bary causes Sclerotinia stem rot of oilseed rape or canola (Martens et al., 1994; Purdy, 1979; Zhao and Meng, 2003). This disease is economically important in the People's Republic of China as oilseed rape is the major oilseed crop and yield losses due to this disease can range from 10% to 80% (Guan, 2011; Ma et al., 2009b). Application of fungicides is the primary method for control of diseases caused by this pathogen (Bardin and Huang, 2001; Lu, 2003; Ma et al., 2009b; Yu and Zhou, 1994). Several fungicides are available but these chemicals are expensive and there are concerns regarding their use as they are hazardous to both humans and the environment. Additionally, some of these fungicides, such as carbendizem and dimethaclon, are becoming ineffective due to the development of fungicide resistance (Fernando et al., 2007; Lu, 2003; Ma et al., 2009a, 2009b; Wang et al., 2015; Yin et al., 2010).

Our long-term goal is to develop a sustainable disease control strategy for *S. sclerotiorum* on oilseed rape based primarily on the use of microbial biological control agents (Hu et al., 2005, 2011; 2013a, 2013b; 2014; Hu et al., 2015). A treatment consisting of *Trichoderma* sp. Tri-1 (Tri-1) formulated with an oilseed rape seedcake fertilizer and rice straw, that was cultivated into field soil prior to sowing oilseed rape, provided control of this disease on oilseed rape in a rice-oilseed rape rotation (Hu et al., 2015). Results from these experiments also suggested that this formulated Tri-1 treatment could be used in integrated control strategies

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containing fungicide sprays. An approach to improving sustainability of disease control is to combine biologically based control tactics with reduced rates of application of chemical pesticides. Using rice – oilseed rape and soybean-oilseed rape rotations prevalent in China, the objective of this study was to determine if combining the formulated Tri-1 treatment with reduced rates of application of pesticide could provide adequate control of *S. sclerotiorum* on oilseed rape.

Trichoderma sp. Tri-1 (Tri-1) was isolated originally from the rhizosphere of an oilseed rape root (*Brassica napus* L. cv. Zhongshuang 9) in a research plot at the Oil Crops Research Institute, Wuhan, People's Republic of China and was obtained from the culture collection of the Plant Protection Laboratory, Oil Crops Research Institute, Wuhan. Isolate Tri-1 is also held in the Agricultural Culture Collection of China (Beijing) as ACCC 32501. This isolate was shown to decompose rice straw and infect sclerotia of *S. sclerotiorum* (Hu et al., 2010). For studies reported here isolate Tri-1 was formulated in 50% oilseed rape seedcake (Wuhan Zhongyou Kangni Technology Co., Ltd., Wuhan), 20% rice straw (removed from an experimental field after grain harvest), and 30% water (*w/w/v*) as described by Min (2008) and Hu et al. (2015).

A total of four field experiments were conducted from October through May in a single year at two research centers essentially as described (Hu et al., 2011, 2013a; 2014). Two of these experiments, using the rice-oilseed rape rotation, were conducted at the Wuxue County Agricultural Technology Generalizing Center, Hubei Province, China. These fields contained a Paddy soil (P, 7.1 mg per kg; N, 124.1 mg per kg; K, 111.6 mg per kg; organic matter, 13.7 g per kg; pH 6.7). Two of these experiments, using the soybean-oilseed rape rotation, were conducted at the Wuhan Caidian District Agricultural Technology Generalizing Center, Wuhan, China. These fields contained a silty-clay loam soil (P, 6.5 mg per kg; N, 117.0 mg per kg; K, 236.2 mg per kg; organic matter, 21.2 g per kg; pH 7.2). All fields contained sclerotia of *S. sclerotiorum* Ss-1 remaining from prior field trials. At each of the four field sites three blocks were arranged in a random complete block design surrounded by a 1-m-wide protective belt of oilseed rape plants. Each replicate treatment plot in each block was 6.6 m × 2 m with ten rows planted at a density of 6 seeds per m and rows spaced 33 cm apart. There were eight treatments at each field site: (*i*) no treatment, (*ii*) the chemical pesticide carbendizem sprayed at 100% the recommended rate, (*iii*) carbendizem sprayed at 75% the recommended rate, (*iv*) carbendizem sprayed at 50% the recommended rate, (*v*) 100 g formulated Tri-1 per plot cultivated into the soil prior to sowing oilseed rape, (*vi*) same as treatment *v* except the chemical pesticide carbendizem was also sprayed at 100% the recommended rate, (*vii*) same as treatment *v* except carbendizem was also sprayed at 75% the recommended rate, and (*viii*) same as treatment *v* except carbendizem was also sprayed at 50% the recommended rate. Carbendizem was applied as a spray (2.0 g [25% a.i., 75% inert material] in 500 mL tap water, with approximately 4.0 mL applied to each plant) when approximately 70% of the oilseed rape plants were flowering. Carbendazim application rates were based on the recommended rate for oilseed rape in this region of China. All treatments had fertilizer (urea, 460 g; superphosphate, 660 g; potassium chloride, 200 g; and borax, 10 g per plot) cultivated into the soil prior to sowing oilseed rape seed. An additional 109 g urea per plot was applied as a top dressing in early January as recommended for this region of China (Su et al., 2011).

At the seven-leaf stage, rows were thinned to 12 plants per row. Five days prior to harvest, 240 plants from each replicate plot were rated for disease incidence (Li et al., 2007). An oilseed rape plant was considered diseased if one third of the branches on the plant contained one or more lesions resulting from infection by *S. sclerotiorum* or if the plant contained a lesion on the caulis (Zhou, 1994). *S. sclerotiorum* was isolated from diseased tissue from random samples to confirm that disease was caused by this pathogen. Mean disease incidence was determined for each treatment replicate, subjected to analysis of variance, and differences among means estimated using least significant difference (LSD) in Proc GLM in SAS ver. 9.3 (SAS Institute, Cary, NC). Disease incidence data from the two field experiments conducted at the Wuxue County field sites ($P = 0.5910$) and the two field experiments conducted at the Wuhan field sites ($P = 0.8421$) were combined prior to analysis ($n = 2$) as there were no significant experiment × treatment effects.

Yield was determined at harvest by sampling 240 plants in each replicate plot for seed dry weight. Seed were weighed after drying in the sun for 2 days. Mean yield per 240 plants was determined, subjected to analysis of variance, and differences among means estimated using LSD in Proc GLM in SAS. Yield data from the two field experiments at the Wuxue County field sites ($P = 0.9988$) and the two field experiments at the Wuhan field sites ($P = 0.9653$) were combined prior to analysis ($n = 2$) as there were no significant experiment × treatment effects.

In experiments conducted at Wuxue County with the rice-oilseed rape rotation, treatments containing 100 and 75% recommended rates of carbendazim as well as all treatments containing formulated Tri-1 significantly reduced incidence of Sclerotinia stem rot on oilseed rape relative to the non-treated control (Table 1). The treatment containing 100% recommended rate of carbendazim resulted in significantly lower disease incidence than other treatments containing reduced rate applications of this pesticide as well as the formulated Tri-1 treatment alone. The treatment containing 50% the recommended rate of this pesticide did not control this

Table 1
Impact of formulated *Trichoderma* sp. Tri-1, chemical pesticide, and their combinations on disease incidence and oilseed rape seed yield in field experiments in a rice-oilseed rape rotation.[a]

Treatment	Mean disease incidence	Mean yield (kg) per 240 plants
Control (no Tri-1 or chemical pesticide)	11.43 A	2.78 A
Carbendazim 100% rate	5.47 EF	3.11 A
Carbendazim 75% rate	8.91 BC	2.88 A
Carbendazim 50% rate	10.34 AB	2.78 A
Tri-1	7.45 CD	2.94 A
Tri-1 + Carbendazim 100% rate	4.51 F	3.19 A
Tri-1 + Carbendazim 75% rate	5.81 DEF	3.05 A
Tri-1 + Carbendazim 50% rate	6.58 DE	2.98 A
LSD	1.79	0.46

[a] For disease incidence and yield per 240 plants treatment values are the mean of two experiments ($n = 2$) each containing three replicates for each treatment. The chemical pesticide carbendazim was applied at 100%, 75%, or 50% the recommended rate at flowering. Tri-1 indicates that a formulation containing *Trichoderma* sp. Tri-1 was cultivated into the soil prior to sowing oilseed rape seed. Repeat experiments were conducted in the same year at a different field location. Means were separated by least significant difference (LSD). Values within a column followed by the same letter are not significantly different ($P \leq 0.05$).