Suppression of the propagation of *Plasmodiophora brassicae* inoculum by early management of volunteer oilseed

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Introduction

Clubroot of oilseed rape (OSR), caused by an obligate biotroph pathogen *Plasmodiophora brassicae*, is a destructive disease and an emerging threat to German OSR production. Besides susceptible host plants, OSR volunteer seedlings as well as Brassica crops and weeds can play a critical role in the maintenance of resting spore populations in infested fields.

The objectives of this study were to: (i) evaluate the optimum timing of post-harvest management of volunteers in the reduction of clubroot incidence and severity; (ii) investigate the effect of timing of mechanical destruction or herbicide application on *P. brassicae* resting spore propagation; and (iii) estimate the effect of postharvest management on clubroot severity in the succeeding oilseed rape crops.

Figure 1. Clubroot symptom

Materials and methods

Winter OSR cv. Ladoga, which is susceptible to clubroot, was used in this study. The isolate of *P. brassicae* was collected from a naturally infested OSR field and was classified as 16/31/31 or P1 on the deferential sets of Buczacki et al. (1974) and Somé et al. (1996), respectively (Zamani-Noor, 2017). Plants were inoculated at growth stage 11-12 by injecting 2 × 1 ml of spore suspension (1×10^7 spores ml^{-1}) into the soil at two locations near the root zone of each seedling. Later on, plants were terminated at two time points after inoculation: early, at 7 dpi, and late, at 21 dpi. Treatments involved mechanical destruction of crops or herbicide application to the foliage. Disease assessment was done by harvesting roots samples at three time points after inoculation (prior to treatment at 7 and 21 dpi and 35 dpi). Samples were visually assessed for clubroot severity based on a scale of 0 to 3. Afterwards, disease incidence (DI) and disease severity index (DSI) were calculated for each treatment. Furthermore, a greenhouse assays were fulfilled by reisolating resting spores of each treatment at 35 dpi and by the subsequent re-inoculation of each extract onto new OSR plants.

Figure 2. Effect of foliar application of glyphosate and mechanical destruction of OSR volunteers (photo is taken at 14 days post inoculation and 7 days after the first application)

Results and Discussion

The results confirm that the presence of OSR volunteers constitutes an important source of inoculum of *P. brassicae*; destruction of OSR plants and volunteers limited the increase of *P. brassicae* resting spores in the soil. Late treated roots, showed significantly stronger disease incidence and severity, bigger clubs and higher numbers of resting spores compared with early treated ones (Table 1).

Table 1 Effect of timing and method of plant termination (7, 21 and 35 days post inoculation; dpi) on clubroot incidence and disease severity index (above) and on root fresh weight (g) and resting spore production inside the root (down) in OSR plants inoculated with *P. brassicae* resting spores under greenhouse conditions.

	Clubroot incidence (%)			Disease severity index (%)		
Treatments	7 dpi	21 dpi	35 dpi	7 dpi	21 dpi	35 dpi
Positive control	10±11.5	100±0.0 b	100±0.0 b	3.3±3.8	80.0±23.7 b	98.0±10.6 b
Early mechanical tr.		25±10.0 a	22±11.5 a		8.3±12.6 a	7.6±3.4 a
Early herbicide tr.		20±10.0 a	20±16.3 a		6.6±5.4 a	6.9±6.3 a
Late mechanical tr.			100±0.0 b			80.0±14.4 b
Late herbicide tr.			100±0.0 b			68.2±28.8 b

	Root fresh weight (g)			Resting spores g $root^{-1}$		
Treatments	7 dpi	21 dpi	35 dpi	7 dpi	21 dpi	35 dpi
Negative control	0.4±0.2 a	1.7±0.7 a	2.9±1.7 a	-	-	-
Positive control	0.5±0.4 a	7.3±2.9 b	16.6±3.3 c	4.4×10^4	2.8×10^5 b	3.0×10^6 c
Early mechanical tr.		0.5±0.2 a	0.5±0.4 a		3.6×10^4 a	3.3×10^4 a
Early herbicide tr.		0.5±0.3 a	0.4±0.5 a		2.2×10^4 a	2.6×10^4 a
Late mechanical tr.			5.5±3.8 b			1.7×10^5 b
Late herbicide tr.			6.4±4.1 b			2.5×10^5 bc

Data are pooled across two runs (i.e., repetitions) and shows means ± standard deviations. Values with different letters within the same column indicate significant differences ($p\leq0.05$) between the treatments; calculated by the Tukey HSD test.

There was a strong correlation between the disease severity index and the root fresh weight and also between the disease severity index and the number of resting spores g $roots^{-1}$ at 35 dpi. The comparison between root fresh weight (g) and the number of resting spores g $roots^{-1}$ revealed the strongest relationship between these two variables (Fig. 3).

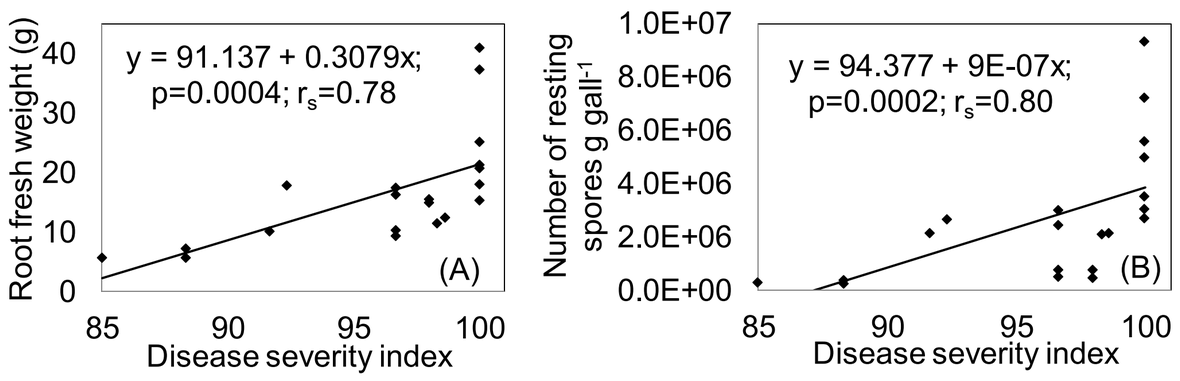

Figure 3. Correlation coefficient of (A) disease severity index and the root fresh weight, (B) the disease severity index with the number of resting spores g gall-1 and (C) root fresh weight (g) with the number of resting spores g $gall^{-1}$ in a susceptible OSR cultivar at 35 days after inoculation. r_s: Spearman's rank correlation coefficient; (p-value ≤0.05).

Reisolation and reinoculation experiment was satisfied by observing symptoms in inoculated OSR plants that were similar to those originally produced. Clubroot incidence and severity were significantly lower in plants inoculated with the spore extraction obtained from early treated roots (up to 85-90 % reduction) (Fig. 4)

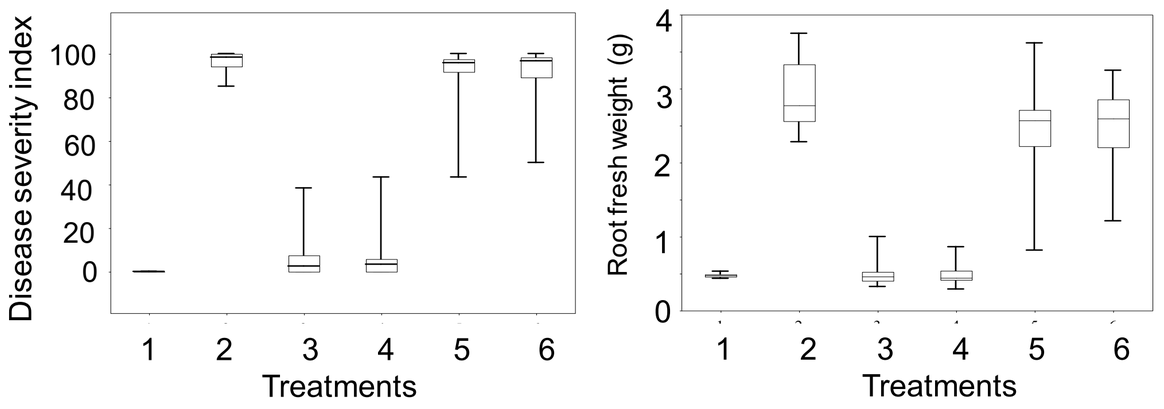

Figure 4. Box-Whisker-Plots of (A) clubroot severity index and (B) root fresh weight of oilseed rape plants which were inoculated with spore suspensions obtained from clubroot-infected roots taken from plants that had been terminated by mechanical destruction or by herbicide application, either 7 or 21 dpi, or that had been left undisturbed to complete development.

Zamani-Noor N, Rodemann B, 2018. Reducing the build-up of *Plasmodiophora brassicae* inoculum by early management of oilseed rape volunteers. Plant Pathology 67, 426–32.