Crop Protection 29 (2010) 599–602

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Hot water treatment prevents *Aphelenchoides besseyi* damage to *Polianthes tuberosa* crops in the Mekong Delta of Vietnam

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ARTICLE INFO

Article history:
Received 15 June 2009
Received in revised form
15 January 2010
Accepted 18 January 2010

Keywords:
Aphelenchoides besseyi
Hot water treatment
Host–parasitic relationship
Mekong Delta
Nematode
Plant disease
Polianthes tuberosa
Vietnam

ABSTRACT

Polianthes tuberosa is a commercially valuable flower crop in the Mekong Delta of Vietnam that is propagated by the harvesting and planting of bulbs. Cultivation of *P. tuberosa* is infected by an endemic *Aphelenchoides besseyi* nematode that damages a high proportion of plants and persists within the bulbs. Here we report on the comparison of hot water and pesticide treatments as control methods to protect *P. tuberosa* from *A. besseyi* damage, and conclude that a hot water treatment consisting of soaking bulbs in water for 30 min at 57 °C is the most efficacious method to produce healthy flowers in a cost effective manner.

1. Introduction

Polianthes tuberosa L., Sp. Pl. (1753) 316 is a bulb crop usually grown for its meter-long flower spikes that produce some 20 fragrant white florets (Huxley et al., 1992; Morris, 1984). In the Mekong delta of Vietnam, *P. tuberosa* is grown commercially and is used mainly in temples, pagodas, at funerals and for worshiping ancestors. Many farmers dedicate up to a quarter of their land to its culture, often in intercropping or rotation with rice or vegetables. The crop is highly valuable, fetching as much as 10 times the value of a rice crop over a given area. Unfortunately, the culture of *P. tuberosa* is a risky business in the Mekong Delta; more often than not, the crop is severely damaged or completely destroyed by a nematode parasite. We previously identified the nematode as *Aphelenchoides besseyi* Christie, and showed that it is an ectoparasite that can persist for several months on the harvested bulbs or dried flowers (Cuc and Pilon, 2007). *A. besseyi* has also previously been shown to be a parasite of *P. tuberosa* in West Bengal, India (Khan, 2004; Khan and Pal, 2001), as well as in Hawaii (Holtzmann, 1968). Surveys have shown that current methods employed by cultivators in the Mekong Delta to try and control infections in *P. tuberosa* mostly rely on air-drying of bulbs for several weeks prior to replanting, and on the application of several types of pesticides of unproven efficacy (Cuc and Pilon, 2007).

A. besseyi is a well-documented parasite that causes "white tip" disease in rice (Fortuner and Orton Williams, 1975; Nandakumar et al., 1975; OEPP/EPPO, 2004). Management of *A. besseyi* often involves soaking of rice seeds in aqueous emulsions of nematicides and subsequent air-drying of the seeds for several days (Hoshino and Togashi, 2000). An alternative control method consists of a hot water treatment of the rice seeds (Fortuner and Orton Williams, 1975; Nandakumar et al., 1975): soaking rehydrated seeds at 57 °C for 10 min kills the nematodes without harming the plant tissue. Indeed, hot water as a control method against nematodes of the genus *Aphelenchoides* has a long history, with the first report dating back to 1935 where it was used to control *A. fragariae*, a parasite of strawberries and begonia chrysanthemum (Christie and Crossman, 1935). More recently, the hot water treatment has also proven efficacious in controlling *A. besseyi* infection on *P. tuberosa* under net house conditions (Khan, 2004). For the present report, we assessed and compared the efficacy and cost-effectiveness of hot water and chemical treatments as possible control methods in greenhouse and experimental field conditions.

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doi:10.1016/j.cropro.2010.01.006

2. Materials and methods

2.1. Pesticides

The following commercial pesticides, with the active ingredient indicated within parenthesis, were used: Actara 25 WG (thiamethoxam 250 g/l kg WG), Basudin 40 EC (diazinon 400 g/l EC), Bemyl 500 WP (benomyl 500 g/kg WP), Confidor 100 SL (imidacloprid 100 g/l SL), Diazan 60 EC (diazinon 600 g/l EC), Marshall 200SC (carbofuran 200 g/l SC), Nokaph 20 EC (ethoprophos 200 g/l EC) and Regent 800 WP (fipronil 800 g/kg WP).

2.2. P. tuberosa harvesting and initial preparation

Infected bulbs (diameter 2–3 cm) were collected from severely infested fields. The bulbs were stored in open air under a roof for one month after which they were cut at their upper end to produce bulbs of 6.5–7 cm in length.

2.3. Nematode extraction and counting

A modified Baermann funnel method was used to extract nematodes from plant tissues (Cuc and Pilon, 2007). Plant tissues were cut into small pieces roughly 2–4 mm in diameter and placed as a 1-cm-thick layer onto a porous tissue supported by a wire mesh (1 mm aperture) glued to the bottom of a plastic ring. The sample was then submerged in tap water in a glass petri dish, but held at 1 cm above the bottom of the dish, and nematodes were allowed to migrate out of the tissue and into the water for 1–2 h. The water was then poured into a beaker, replaced with new water, and the nematodes allowed to emerge for another 1–2 h, producing another sample. Five samples were produced in this way, with one exception: for the fifth and final sample, nematodes were allowed to emerge during an overnight incubation. The pooled samples were mixed by stirring, 1 ml samples were examined under the microscope, the nematodes counted and the result extrapolated to the whole extract.

2.4. P. tuberosa bulb treatments

During the optimization of the hot water treatment, bulbs held within a mesh in a temperature-controlled water bath set at temperatures of 50 °C, 55 °C, 56 °C, 58 °C, and 60 °C were treated for durations of 15, 30 and 45 min. The derived optimized hot water treatment consisted of immersing bulbs held within a mesh in five volumes of water at 57 °C, incubating for 15 min by which time temperature dropped to 53–54 °C, then readjusting the temperature to 57 °C by the addition, while stirring, of 2–3 bulb volumes of boiling water and a further incubation of 15 min. For pesticide treatments, bulbs held in a mesh were soaked in five volumes of diluted pesticides at ambient temperature for periods of 6, 24 or 48 h. Following the hot water or pesticide treatments, the bulbs were dried for two days in open air under a roof then examined visually: only bulbs with no signs of rotting were scored as healthy.

2.5. Small-scale field experiment

A 160 m^2 field in Vi Thanh district, Hau Giang province was divided into 4 rows of 4 plots to produce 16 plots of 8 m^2 (1.2×6.6 m). The field had previously supported rice, had no history of *P. tuberosa* cultivation and was not fumigated prior to the start of the experiment. Four treatments were tested: control (untreated), hot water (57 °C for 30 min), diazinon (5.63 ppm for 6 hours) and fipronil (200 ppm for 48 hours). Each row of 4 plots served as one replicate of the experiment and each plot contained 198 bulbs from one treatment, planted as 3 lines of 22 groups, with 3 bulbs per group. The distribution of the four treatments among the four plots in each replicate was randomized, and the experiment contained four replicates for a total of 792 bulbs for each treatment tested. The field was monitored for 16 weeks, weeds were removed manually twice a week, 1.2 tons of organic fertilizer (straw plus animal fertilizer) was applied to the field at the time of planting, and 60 kg/ha of NPK (16-16-8) fertilizer was added at 15, 30 and 45 days post-planting. Watering was done every two days during dry periods. Nematodes were counted in a sample of 40 bulbs prior to treatments, and the number of living plants per plot as well as the number of shoots per plant were counted 16 weeks after planting. The shoots were also inspected for presence of symptoms of *A. besseyi* infection, and the density of nematodes per plant was determined in 20 randomly chosen plants for each treatment and replicate (thus 80 plants scored per treatment).

2.6. Full-scale field experiment

The experiment was conducted in a 554 m^2 field belonging to a local farmer in Cai Lay district, Tien Giang province between 1 October 2007 and 2 March 2008. This field had recently supported a crop of *P. tuberosa* severely infected with *A. besseyi* from which >40% of the plants exhibited damage due to infection, which is typical of fields where *P. tuberosa* has been cultivated for several seasons (Cuc and Pilon, 2007). The field was divided into four replicates separated from each other by 1 m. Each replicate included four rows of 20.8 m^2 (26×0.8 m) separated from each other by 0.5 m. Each row of each replicate was planted with 800 bulbs in 80 rectangular mounds, each mound being planted with 2 lines of 5 bulbs. The bulbs were obtained from a severely infected field where over 70% of the bulbs were infected with *A. besseyi*, with a count of nematodes per bulb before treatment of 45.8 (SE = 12.9) as determined from scoring 40 bulbs. Four treatments were tested: control (untreated), hot water (57 °C for 30 min), diazinon (5.63 ppm for 6 h) and ethoprophos (200 ppm for 48 h). Weeds were removed manually twice a week, and the plants were watered every two days during dry periods. The number of healthy and diseased plants was determined at 12, 14, 16 and 20 weeks post-planting, and the disease severity on the infected plants was classified using four categories (see Fig. 1A): DS1 (normal plant height, slightly short panicle compared to healthy plants, some flowers fail to open); DS2 (short plant height, short panicle, many flowers fail to open); DS3 (very short height, very short panicle, many flowers fail to open); DS4 (very short height, very short panicle, flowers buds fail to develop, severe browning of stems, leaves and buds).

2.7. Cost–benefit analysis

The costs or cultivation and pesticide uses, yields and income were averaged from several reference fields in the Mekong Delta following surveys by the local farmers (Cuc and Pilon, 2007). Typically, marketable flowers were harvested every second day and sold to the market, and all transactions were recorded.

2.8. Statistical analysis

The statistics analysis software JMP® 7.0 produced by SAS was used in all statistical analyses. Comparisons of means were done using Student's *t* test, and nematode count data was normalized by log-transformation prior to statistical analysis. For the analysis of the full-scale field experiment, data from all the plants in each of the four replicate were summed as one measurement, providing four measurements per treatment for each time point.