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Spatial distribution of *Heterodera trifolii* in Chinese cabbage fields

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ABSTRACT

Although *Heterodera trifolii* is commonly known as the clover cyst nematode, recently the nematode has been identified as a serious menace for Chinese cabbage growers in highland areas in Korea. Soil samples were collected from two Chinese cabbage fields highly infested with *H. trifolii* in highland areas of Korea, Jungsun and Samcheok, in 2014 and 2015, respectively. A total of 777 (2 × 2 m sampling area) and 414 (5 × 5 m area) soil samples were collected from Jungsun and Samcheok, respectively. The total cysts, cysts with eggs, number of eggs, and empty cysts were calculated for each sample. Distribution patterns for these variables were characterized using spatial analysis by distance indices (SADIE) and variogram model analysis. The aggregation index for cysts with eggs was higher in Jungsun (89.32) than Samcheok (3.63), which indicated that the cyst population density was higher. However, the spatial association of total cysts versus cysts with eggs was higher in Samcheok. The Gaussian model showed reasonable independent range of the nematode in Jungsun and Samcheok to be approximately 53.66 m and 48.54 m, respectively. The model suggested that each nematode sample should be taken at least 50 m apart in the given areas. Inclusion of this distribution pattern may significantly minimize the number of samples in future sampling methods, which could save time and labor, and initiate management practices by elucidating spatial variability factors that influence crop yield.

Introduction

Heterodera trifolii belongs to the large group of cyst-forming nematodes with morphological characters close to those of the sugar beet cyst nematode *Heterodera schachtii*. *H. trifolii* is a well-known and important pathogen of clovers, including sugar beets, causing severe economic losses (Mass and Heijbroek, 1982). This nematode is distributed worldwide (Mulvey, 1972) and it is known to survive on a relatively wide range of hosts. The host range of *H. trifolii* extends to at least 110 species, although it mainly subsists on white and red clover, spinach, strawberry, cabbage, and radish plants (Evans et al., 1998). Host suitability and susceptibility records of this nematode are conflicting and most probably caused by varietal differences in host species (Mulvey, 1972). Host preference of *H. trifolii* also varies with nematode race and isolates (Mass and Heijbroek, 1982). The populations can easily survive on alternative hosts and weeds occurring between crops (Perry and Gaur, 1996). Crop losses caused by *H. trifolii* have only been quantified for clover, beets, and other leguminous crops (Perry and Gaur, 1996). However, no information is available on Chinese cabbage or any other crop in Korea.

Chinese cabbage, *Brassica rapa chinensis* L., a high-valued economic highland crop (Kim et al., 2014) that is mainly used for kimchi production. Chinese cabbage is the third most produced crop in Korea (KREI, 2012). Chinese cabbage grown in highland areas is favored because of the local environmental conditions and it has a high market value across the country. However, the main production constraints for cabbage include cyst nematodes (*H. schachtii* and *H. trifolii*), insect pests, and diseases caused by various pathogenic microbes (Seo et al., 2009; Mwamula et al., 2018). Thus, the economic damage caused by *H. trifolii* is a major production setback, which calls for immediate determination of reliable control and management strategies.

Because of the higher elevation and sloped landscape in the Gangwon-do Province, the nematode has been disseminated to the lower elevations by surface runoff and agricultural equipment (Kwon et al., 2016). Because clover cyst nematodes have a relatively wide host range, and their management involves an integrative approach with different strategies, including prevention, cultural practices, resistant cultivars, trap crops, physical methods, and chemical control (Turner and Rowe, 2006). The challenge of controlling nematode pests is becoming more difficult in these areas because of unknown population

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dynamics. Furthermore, few studies have been conducted to evaluate the effectiveness of chemical control methods (Lee et al., 2018) and other management strategies (Kwon et al., 2016), and research has been limited to cultural and chemical control.

It is crucial to elucidate population density, type, and damage potential before choosing and implementing a feasible management practice (Hill, 1988). Knowledge of the spatial distribution, ecology, and genetics of any pathogen population can increase our understanding of species dynamics and accelerate field management of pathogen populations by improving crop resistance deployment strategies (van Bezooijen, 2006). The most studied examples of natural control of plant-parasitic nematodes involve field distribution and declines in population number. The field distribution of nematodes has been described as aggregated (Ferris and Wilson, 1987), which indicates that nematode data are spatially dependent. Considerable variability in the spatial distribution patterns of the species composition and abundance of plant parasitic nematodes has been previously reported from agricultural fields (Robertson and Freckman, 1995). Aggregated nematode data could possibly remove a substantial degree of uncertainty from estimates of population size and significantly add to the effort required for comprehensive measurement (Robertson and Freckman, 1995).

To quantify the spatial pattern of distribution, classical spatial pattern analyzing methods are focused on the among-sample count variance and mean without spatial locations (Taylor, 1984; Liebhold et al., 1991). This produces certain undesirable effects which are sometimes fail to differentiate different spatial patterns (Jumars et al., 1977; Midgarden et al., 1993; Perry et al., 1999) and their descriptions of spatial pattern are highly dependent on the size of sample units (Sawyer, 1989). Moreover, classical methods should be calculated under assumption that the individual sample counts are spatially independent without consideration of the spatial continuity (Perry et al., 1999; Kim et al., 2001). In this study, the spatial distribution by distance indices (SADIE) and variogram model analysis were used to analyze and explain the spatial distribution pattern for *H. trifolii* in Chinese cabbage fields. The SADIE methodology has been used in a wide range of research disciplines, such as plant-soil interactions (Maestre et al., 2003; McGraw and Koppenhofer, 2010). However, variability in the spatial distribution of the nematode population is possibly best explained by using geostatistical tools and once the spatial distribution pattern is known for a certain area, map values at unsampled locations can be easily predicted (Felicitas et al., 2016). Studies have shown that the direction of spatial variation of plant-parasitic nematodes was related to the direction of tillage, but not to other factors such as soil type or weed distribution (Donald et al., 1999). However, little or no studies have been conducted on the spatial distribution of these nematodes in highland areas of Korea. Almost all of the existing knowledge is based on studies concerning their control, dissemination, and general biology. Such studies are indispensable for the development of sampling plans, aimed at the application of an integrated management program (Taylor, 1984; Giles et al., 2000). The study of spatial distribution of this nematode may help to control them in Chinese cabbage through a better understanding of the environmental factors driving population dynamics. In addition, using spatial distribution data, application of nematicide can be reduced to target areas where the nematode is above threshold, and thus reduce environmental contamination and decrease production costs (Davis et al., 2013; Mueller et al., 2010). Therefore, effective management would be enhanced given a better understanding of the spatial and temporal dynamics of the incidence of *H. trifolii*. The results from this study will enhance the ability to identify total population variance of the given fields that is spatially aggregated and to identify the minimum sampling range scale.

Given the above, there is a strong need to study the spatial distribution pattern of *H. trifolii* total cysts, cysts with egg, eggs per cysts, and empty cysts under field conditions using geostatistical tools. The individual nematode distributions occasionally corresponded to soil moisture and nitrate content but not to soil resource pattern within small-scale (i.e. < 4.5 m distance), however, in large-scale (> 4.5 distance), spatial pattern of nematode population trends persisted in same taxa regardless of sampling season (Ettema et al., 1998). Thus, we hypothesized that the spatial distribution of the nematodes is sufficiently structured and time invariant to support the use of nematode management practices in infested fields. This study was designed to achieve the following objectives:

1. To evaluate the spatial structure of nematode populations from highland areas.
2. To find the aggregation pattern of the cyst population for proper sampling optimization.
3. Initiate management practices given knowledge of spatial variability factors.

Materials and methods

Study site and soil sampling

The study was conducted in two different Chinese cabbage fields that were highly infested with nematodes in 2014 and 2015 (Jungsun and Samcheok, respectively) in the Gangwon-do Province, Korea. Study area in Jungsun (37° 26′ 23.20″ N and 128° 51′ 23.80″ E) and Samcheok (37° 22′ 8.84″ N and 128° 59′ 58.78″ E) was 0.78 ha and 0.41 ha respectively and both fields were conventionally tilled for Chinese cabbage. During early autumn 2014, a total of 770 soil samples were obtained from Jungsun. Each sample was taken within a 2 m wide × 2 m length plot to a depth of approximately 30 cm using a transplanting trowel. Soil samples from Samcheok (414 samples) were obtained during spring 2015. Each sample was taken within a 5 m wide × 5 m length plot to a depth of approximately 30 cm using a transplanting trowel. All the collected samples were taken to the laboratory for further analysis.

Nematode extraction and counting cysts

A subsample of 100 g soil was taken from each collected sample for nematode extraction. Sieving (20- and 60-mesh sieve [850 and 250 μm, respectively]) was used to obtain cysts. Only 60-sieve particles were filtered with Whatman no. 100 filter paper. Then, the filter paper was placed in a 6 cm diameter petri dish, and placed under a stereomicroscope (Nikon SM2 1000, Japan) to successfully isolate and count the total number of cysts and cysts with eggs. Five randomly chosen healthy cysts (undamaged, with eggs) were transferred into a small vial with 5 mL water. The cysts were sonicated at 8000 rpm using a Polytron PT 1300D sonicator (Kinematica AG, Switzerland). Sonicated eggs containing water were transferred to a petri dish and placed under a microscope to count the number of eggs.

Data analysis

Cysts with eggs, the number of eggs per cyst, and total number of cysts were initially analyzed by descriptive statistics, including means and standard deviations generated using MS Office Excel 2016. Histograms and cumulative distribution functions were analyzed to compare the frequency distribution of cysts, eggs per cyst, and total number of cysts between fields.

Spatial distribution patterns analysis of H. trifolii

In this study, the spatial distribution pattern analysis of *H. trifolii* from two Chinese cabbage fields were evaluated in the following order: first, spatial distribution analysis of each stage of *H. trifolii* was evaluated by aggregation indices and spatial red-blue plots analysis of SADIE. Second, spatial relationship among different stages of *H. trifolii*.