

Effect of Fluctuating Temperature on the Damage Potential of *Heterodera schachtii* on Chinese Cabbage

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I. Introduction

Chinese cabbage, *Brassica rapa chinensis* L. is a highly valued economic crop in Korea (Kim *et al.*, 2014). It is ranked as the most important processed food stuff and a highly important native side dish in form of Kimchi (FAO, 2013; Park *et al.*, 2014). Cabbage production is mainly done in the highland areas of Gangwon-do and the main production constraints include; insect pests, diseases caused by various pathogenic microbes and most recently the intercepted sugarbeet cyst nematode, *Heterodera schachtii* (Lee *et al.*, 2013; Kim *et al.*, 2014). *Heterodera schachtii* is a well-known important pathogen of sugar beet causing severe economic losses (Griffin, 1987; Hafez & Seyedbagheri, 1997). The pathogen is known to survive on a relatively wide host range within 95 genera. Thus, populations can easily survive on alternative hosts and weeds occurring between crops (Steele 1965; Perry and Gaur, 1995). Additionally, the pest can also survive extended periods in the absence of a host in form of cysts formed when mature females die and the cuticle becomes tanned into a tough protective envelope encasing approximately 500 to 600 eggs (Turner and Rowe, 2006). Infective juveniles hatch from the enclosed eggs under the influence of root exudates from host plants (Kabir *et al.*, 2015).

However, hatching does not depend on presence of root exudates alone but the process is also determined by changing environmental conditions during the whole growth period of the host. Temperature is a major factor regulating the development of beet cyst nematodes (Griffin, 1981a; Trudgill, 1995). Under favorable environmental conditions, *H. schachtii* juveniles can hatch under limited influence of host plant exudates (Perry and Gaur, 1995). It is noted that the optimum temperature for infective juvenile hatching is 25°C and lower temperatures have a negative influence on the hatching of *H. schachtii* (Kabir *et al.*, 2015). Chinese cabbage in Korea is planted after winter season when temperatures are still below 15°C, far below the optimum hatching temperatures of *H. schachtii*. However, temperatures continuously increase over the spring season to the summer period to levels which are

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deemed favorable for the reproduction of SBCN (KMA, 2011).

However, it is important to note that in the highland areas of Korea, there are various micro-climates where temperature fluctuations are distinct from one place to another. During cabbage growth period in the highland areas of Taebaek, temperatures fluctuate between 15 and 22°C while in Haenam, another main cabbage producing area, temperatures fluctuate between 22 and 11°C from planting to harvesting period (Korea Meteorological Administration, Retrieved 2011-05-04). The study therefore aimed at evaluating the effect of fluctuating temperatures on the reproduction and damage potential of SBCN on Chinese cabbage in the highland areas of Korea. This was done under controlled laboratory conditions by mimicking the temperature fluctuations of the cabbage growing highland areas of Korea.

II. Materials and Methods

2.1. Planting Chinese cabbage

30 days old Chinese cabbage plants (variety: Chungwang) were used for this experiment. Chinese cabbage plants were planted in a plastic pot (size 10.5 × 13.5 cm) was filled with 800 g autoclaved soil to ensure no contamination of other soil nematodes. After planting the cabbage, the next day all the pots were inoculated with 160 juveniles per pot. All the plants were planted in 19th January 2016. Two different named growth chamber were set to test the effect of fluctuating temperature. Each had 8 replications.

2.2. Preparation of growth chamber

Two growth chamber (Vision 3250 Bi, Korea) were prepared for this experiment. First one was named as Taebaek which initial temperature was set at 16.7°C and in every 10 days the temperature had been changed to 17.8, 18.8, 19.9, 20.6, 22°C. The second one was named as Haenam, which initial temperature was 21.5°C C and in every 10 days the temperature had been changed to 19.4, 17.4, 15.7, 13.4, 11. 5°C. Temperature was increased by analyzing the last five years' meteorological data.

2.3. Measuring the leaf and weight of plant

Only first four leafs from the ground were measured by length and width in cm with measuring scale. At the same time, plant body weight and root weight were also counted.

2.4. Counting cysts

Cysts were counted after 60 days of inoculation in the pots. First the foliar part of the plant was cut from the ground and then the soil with root was transferred in a jar. Then, the soils were washed by tap water very carefully to separate the root. This part was done with extra care so that maximum number of roots can be obtained intact or undamaged. After that, jars water was passed through using 20, 60 and 400 mesh sieves. Cysts of 60 mesh sieve then filtered with Whatman no. 100 filter paper to remove the water content. Then the filter paper was taken in a petri dish and placed under Nikon SM2 1000 electron microscope to count the female, brown and the total cysts.

2.5. Counting eggs

After selecting 5 healthy cysts from previously counted cysts, are transferred to a small vial with 1.5 ml water and then sonicated in 700 rpm by using Polytron PT 1300D sonicator (Kinematica AG, Swizerand). Then the sonicated water was transferred again into a petri dish to count the number of eggs.

2.6. Counting juveniles

Counting juveniles was followed by the continuation of cysts. During the process of collecting cysts from the pots soil, we used three different sieves of 20, 60 and 400 mesh. The substrates from 400 sieve were transferred into a funnel tube which was already prepared by placing two KIMTECH laboratory tissue on the top of the funnel and the funnel tube was blocked by a clip to avoid water leaking. After 24hour, the tube water which contain the juveniles were collected in a petri dish and placed under Nikon SM2 1000 electron microscope to count the number of juveniles.

2.7. Statistical analysis

All the statistical tests were done by using SAS 9.4.1.

III. Results

3.1. Nematode population

The number of brown cyst, female cyst total cyst, eggs and juveniles were counted after 60 days of inoculating the plants. Results showed that, in both areas the number of brown cyst and female cysts were found in very low number and the data sets were not statistically significant. However, Taebaek recorded highest number of eggs/cyst which was almost 20 times higher than Haenam eggs (df= 1, F = 37.96, p=0.0001). and the number of juveniles were also recorded higher in Taebaek. (df=2, F=1.1, p=0.03).

3.2. Number of leaf

The number of leafs for both Taebaek and Haenam were analyzed and result showed that the leafs from Taebaek were highly significant (df= 1, F=10.89, p=0.005) at 5% level by Duncan test.

3.3. Weight of Chinese cabbage

Interestingly, total body weight of the infected cabbage plants of Taebaek showed less yield losses compared to Haenam. And the results were statistically significant at 5% level by Duncan test where df= 1, F=6.80, p=0.02.

Acknowledgement

This research was supported by a grant from Rural Development Administration (Project Code PJ010774), Republic of Korea.

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