

[Original Article]

Effect of temperature on the development of *Pratylenchus kumamotoensis*

Kenta Uesugi^{1,*} and Hideaki Iwahori²

Development of *Pratylenchus kumamotoensis* eggs was observed in distilled water at 15–35°C. Egg developmental duration was shortest at 32.5°C (5.6 days) and longest at 15°C (28 days). Base temperature and thermal constant estimated from data between 20°C and 30°C were 11.8°C and 108 degree-days, respectively. Population development from a single female inoculated to chrysanthemum was observed at 20, 25 and 30°C. *Pratylenchus kumamotoensis* reproduced bisexually and the number of total progeny from the inoculated female sharply increased 15–18 days after inoculation (dai) at 30°C, 24–27 dai at 25°C and 40–44 dai at 20°C, indicating onset of oviposition by next generation females. These results indicated that *P. kumamotoensis* is closer to tropical or subtropical plant-parasitic nematodes in thermal requirements rather than to temperate species such as *P. penetrans*. Nematol. Res. 46(2) 59–63 (2016).

Key words: base temperature, chrysanthemum, lesion nematode, thermal constant.

INTRODUCTION

Pratylenchus kumamotoensis Mizukubo *et al.* is a nematode pest of chrysanthemum, causing root lesions, defoliation of lower leaves and stunting of stems (Mizukubo *et al.*, 2007; Sugimura and Kawasaki, 2008). The species was a dominant lesion nematode in chrysanthemum fields in the Kyushu and Okinawa region of Japan (Uesugi *et al.*, 2009). Another major chrysanthemum nematode pest, *P. penetrans*, was also distributed in this region, and some fields are infested with both species. Symptoms caused by the two species are similar to each other (Kobayashi, 1995; Sugimura and Kawasaki, 2008). However, the host range of *P. kumamotoensis* is narrow compared to polyphagous *P. penetrans*, while the reproductive rate of *P. kumamotoensis* on chrysanthemum was considerably higher than *P. penetrans* (Uesugi *et al.*, 2011). These data suggested that chrysanthemum nematode control in southern Japan needs to target the two *Pratylenchus* species with rather different host range and reproductive potential.

In addition to reproductive characteristics, a possible difference between *P. kumamotoensis* and *P. penetrans*

is thermal requirements. *Pratylenchus penetrans* is a temperate species with low base temperature for development (Mizukubo and Adachi, 1997). Actually, *P. penetrans* is regarded as an important pest species in relatively cool areas of Japan (Gotoh, 1974). On the other hand, *P. kumamotoensis* is dominant in the southern Kyushu and Okinawa region, overlapping the distribution of important tropical or subtropical species such as *M. incognita* and *P. coffeae* (Koga, 1992; Teruya, 1992). *Pratylenchus kumamotoensis* could be adapted to higher temperature conditions than *P. penetrans*. Temperature and the rate of nematode development are usually linearly related (Trudgill, 1995). Base temperature, which is the lower temperature limit for development, is estimated by the temperature axis intercept of the linear regression for developmental rate at different temperatures. Thermal constant, which is measured as the reciprocal of the slope of the linear regression line, can be used to estimate the duration of development at a certain temperature. Data on these thermal characteristics of pest nematodes are important to predict their generation time, population dynamics, and resulting crop damage.

The purpose of this study is to elucidate base temperature and thermal constant of *P. kumamotoensis* development, which gives insight into temperature adaptation of the species. To calculate base temperature of the species, we examined the duration of the egg stage in distilled water. We also conducted time-course

¹ Kyushu Okinawa Agricultural Research Center, NARO, 2421, Suya, Koshi, Kumamoto 861-1192, Japan

² Faculty of Agriculture, Ryukoku University, 1-5 Yokotani, Setaocho, Otsu, Shiga 520-2194, Japan

*Corresponding author, e-mail: uesugik@affrc.go.jp

observations of population development from single females in chrysanthemum roots to examine the effect of temperature on the duration of a life cycle.

MATERIALS AND METHODS

Effect of temperature on egg developmental duration of *P. kumamotoensis* (Experiment 1):

An isolate of *P. kumamotoensis*, which was derived from a single female originally collected from the rhizosphere of chrysanthemum in Kanoya City, Kagoshima Prefecture, Japan, was used. Nematodes were maintained in chrysanthemum 'Jimba' pot culture in a greenhouse and extracted from chrysanthemum roots using a blender-Baermann funnel technique. A gravid female was picked with an insect pin and put in a 2 cm diameter glass dish filled with distilled water. The dish was kept in an incubator at 25°C and checked for oviposition later in the day (1–7 h after pickup) and on the next day (14–19 h after pickup). If oviposition was observed, the female was removed from the glass dish. Then, the dishes containing egg(s) were placed in an incubator (Bio multi incubator LH-30-8, Nippon Medical & Chemical Instruments Co., Ltd.) at the test temperatures (Table 1). The dishes were replenished with distilled water as needed and observed two to three times in a day at 20.0–35.0°C or one to two times in a day at 15.0°C. The observation was continued until hatch was not observed in three consecutive days after peak of hatch at 20.0–35.0°C or continued to 40 days at 15°C. Egg developmental duration was calculated from estimated oviposition time (midpoint between the observation of laid egg and the previous observation) and estimated hatching time (midpoint between the observation of hatched juvenile and the previous observation). Observation at each temperature was examined twice

(trial 1 and 2). To estimate base temperature (developmental zero, °C) and thermal constant (effective accumulative temperature, degree-days), we conducted regression analysis of developmental rate (= 1/egg developmental duration) data from 20.0 to 30.0°C, where decrease in the egg hatch rate (Table 1) or developmental rate (Fig. 1) was not observed. All data from two trials were pooled in the regression analysis. Statistical analysis was conducted using JMP 12 (SAS Institute).

Effect of temperature on life cycle duration of *P. kumamotoensis* (Experiment 2):

Duration of the nematode life cycle in chrysanthemum roots was estimated from time-course change in a number of progenies from a single female. Nematode isolate and culturing methods were the same as experiment 1. Nematodes were extracted from soil of the pot using a Baermann funnel technique. Approximately 7 cm long cuttings of chrysanthemum 'Jimba' were put in a beaker filled with tap water at room temperature until a few centimeter-long roots emerged. A single mature female of *P. kumamotoensis* was placed on the root surface of a cutting on a 9 cm diameter petri dish. The root was then adequately covered with autoclaved sand and moistened by tap water. After overnight incubation at 25°C, roots of the cutting were washed to remove females that failed to penetrate roots. Then, the cutting was planted in a glass vial (3 cm diameter × 7.5 cm height) covered with autoclaved sand and kept in an incubator at test temperatures, 20, 25 or 30°C. The day of transplanting was defined as day of inoculation (= 0 days after inoculation (dai)) in this experiment. Liquid fertilizer (NPK = 0.006% : 0.01% : 0.005%) was added at 1.0 ml (25 and 30°C) or 0.5 ml (20°C) to each cutting weekly.

Table 1. Egg duration (days) of *Pratylenchus kumamotoensis* under different temperature conditions.

Temperature (°C)	Trial 1			Trial 2		
	Number of eggs	Hatched (Hatching rate)	Egg duration ^a (days)	Number of eggs	Hatched (Hatching rate)	Egg duration ^a (days)
35.0	101	7 (7%)	7.3 ± 0.30	80	8 (10%)	6.7 ± 0.45
32.5	80	64 (80%)	5.6 ± 0.05	46	32 (70%)	5.7 ± 0.08
30.0	103	84 (82%)	6.1 ± 0.05	45	36 (80%)	5.8 ± 0.08
27.5	98	67 (68%)	6.8 ± 0.07	49	46 (94%)	6.8 ± 0.07
25.0	101	67 (66%)	8.3 ± 0.08	48	42 (88%)	7.6 ± 0.09
22.5	80	58 (73%)	9.9 ± 0.11	53	45 (85%)	10.0 ± 0.10
20.0	89	63 (71%)	13.9 ± 0.11	50	37 (74%)	13.0 ± 0.14
15.0	80	17 (21%)	28.0 ± 0.53	77	24 (31%)	26.7 ± 0.36

^a Mean ± SE.

Cuttings were sampled every 4 days from 4 dai to 44 dai at 20°C, every 3 days from 3 dai to 30 dai at 25°C, and every 3 days from 3 dai to 24 dai at 30°C. Root samples were stained by the NaOCl-acid fuchsin-glycerin method (Byrd *et al.*, 1983) to count eggs and vermiform nematodes (adults and juveniles) inside roots. Stained root samples were directly observed under stereomicroscope at magnifications up to $\times 115$. When counting was difficult due to high nematode or egg density, root samples were dissected under a stereomicroscope to spread nematodes and eggs inside the root on a glass slide and observed under a light microscope at magnifications of $\times 60$ or $\times 150$. Presence of vulva or spicule in hatched nematodes was checked whenever possible. The number of total progeny from an inoculated female was calculated as follows; total progeny = eggs + hatched nematodes inside roots, where hatched nematodes = number of total vermiform nematodes in roots - 1. Chrysanthemum samples without nematodes inside roots were discarded. We repeated the inoculation-sampling trial until at least 10 nematode-positive samples (maximum 17 samples) were obtained for each combination of temperature and incubation period. Nematodes in sand were not examined.

To reduce the effect of low fertility females, we sorted each data set by the number of total progeny, and

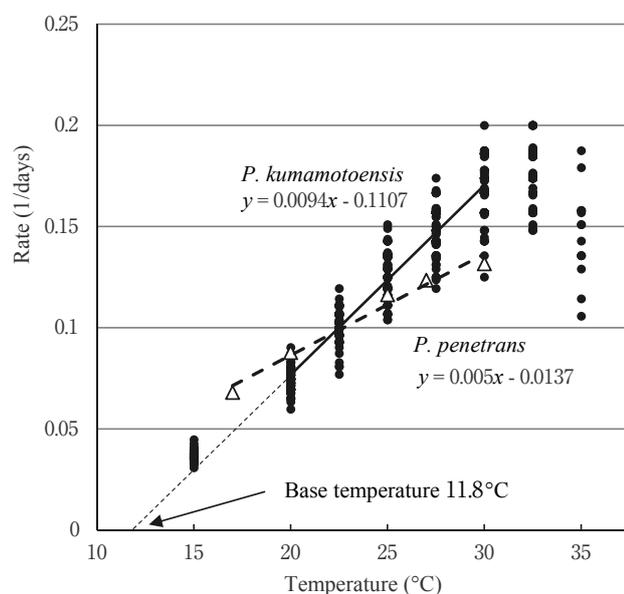


Fig. 1. Relation between temperature and development rate of egg of *Pratylenchus kumamotoensis* (black circle) and *P. penetrans* (white triangle; redrawn from Mizukubo and Adachi, 1997).

All data from two trials are plotted. For regression analysis of *P. kumamotoensis*, data from 20°C to 30°C were used (solid line with extrapolation of thin dashed line).

used the upper half of the data set (rounded up for odd sample number). For example, the top 6 of 11 samples were used in the trial for 9 dai at 30°C.

RESULTS

Experiment 1:

Most females laid only one egg in distilled water, but occasionally two or three eggs were laid. Developmental time and hatching rate are shown in Table 1. Hatching rates were 66% or higher between 20°C and 32.5°C, whereas it decreased to 7–31% at 15°C and 35°C. Average egg developmental duration was shortest at 32.5°C and increased at higher or lower temperatures up to 28 days at 15°C. There was a significant difference between egg developmental durations of two trials at 15°C, 20°C, 25°C, and 30°C (Wilcoxon rank-sum test, $P < 0.05$). However, even in these cases, the differences in the means were small, at most 1.3 days at 15°C. Developmental rate linearly increased with temperature, plateaued at 30–32.5°C, and clearly declined at 35°C (Fig. 1). The base temperature and the thermal constant (degree-days) estimated from combined data of two trials between 20°C and 30°C were 11.8°C and 107 degree-days, respectively (regression equation: $y = 0.0094x - 0.1107$, $R^2 = 0.91$, $P < 0.01$, $y =$ developmental rate, $x =$ temperature) (Fig. 1). The regression line for egg developmental rate of *P. kumamotoensis* intersects that of *P. penetrans* (data from Mizukubo and Adachi, 1997) at 22.0°C.

Experiment 2:

The average of the upper half of data for total progeny, number of eggs and hatched nematodes are shown in Fig. 2. Eggs were observed at the first observation in three tested temperatures indicating oviposition started between 0 dai and 3 dai (30°C and 25°C) or 4 dai (20°C). From these results, oviposition days were set as 1.5 dai at 25°C and 30°C, and 2 dai at 20°C. During the first 9 days at 25°C and 30°C, and 16 days at 20°C, the number of total progeny linearly increased due to an increase of eggs deposited by the inoculated female. After this first increase, the number of eggs decreased as hatched nematodes increased, resulting in a reduced rate of increase for total progeny (9–15 dai at 30°C, 9–24 dai at 25°C, and 16–40 dai at 20°C). Progenies of the inoculated females were almost always observed near the female, where the root showed a small lesion symptom. Offspring with spicule or vulva were first observed 15 dai at 30°C, 21 dai at 25°C, and 36 dai at 20°C. After the occurrence of these matured nematodes, the number of total progeny sharply increased

again, most likely due to onset of oviposition by the next generation females. This fluctuation pattern indicated the onset of oviposition by the next generation females was between 15 dai and 18 dai at 30°C, between 24 dai and 27 dai at 25°C, and between 40 dai and 44 dai at 20°C. From these results, days for oviposition by the next generations are set as 16.5 dai at 30°C, 25.5 dai at 25°C and 42 dai at 20°C. The predicted generation time, from oviposition of the first generation to that of the next generation, at each temperature were 15 days at 30°C, 24 days at 25°C and 40 days at 20°C. The base temperature and the thermal constant were 14.3°C and 240 degree-days, although the regression was not statistically significant ($y = 0.0042x - 0.0597, R^2 = 0.99, P = 0.07$).

DISCUSSION

Thermal characteristics of plant-parasitic nematodes have been studied mainly in economically important root-knot and cyst nematodes (e.g. Griffin, 1988, Madulu and Trudgill, 1994). Although there are a number of studies on life cycle of *Pratylenchus* spp., few studies estimated the base temperature and thermal constant (Mizukubo and Adachi, 1997; Umesh and Ferris, 1992). This is possibly due to difficulty in continuous observation of migratory lesion nematodes in roots and in determination of completion of one generation in a mixed-stage population. In this study, we examined the egg developmental duration to clarify the effect of temperature on development of *P. kumamotoensis*. Embryogenesis is a differentiation process which needs no food supply and is easy to observe, even in migratory endoparasitic nematodes. Furthermore, study of the egg stage has advantages in shorter and less labor-intensive experiments than study of the life cycle. Trudgill (1995) suggested the possibility that base temperatures determined for the egg stage are similar to those for the life cycle. The base temperature of the egg stage of *P. kumamotoensis* (11.8°C) was closer to tropical and subtropical plant-parasitic nematodes, e.g. *M. incognita* (11.4–11.7°C), *M. javanica* (12.7–12.9°C) and *M. arenaria* (10.1°C), rather than to temperate nematodes, e.g. *G. rostochiensis* (5.9–6.3°C) and *P. penetrans* (2.7°C) (Mizukubo and Adachi, 1997; Trudgill, 1995, Tzortzakakis and Trudgill, 2005). The regression line for egg developmental rate of *P. kumamotoensis* intersects that of *P. penetrans* at 22.0°C, indicating *P. kumamotoensis* has a shorter developmental time than *P. penetrans* above this temperature. Such a relationship is comparable to that observed between a tropical and temperate species of root-knot nematode, *M. javanica* and *M. hapla* (Trudgill, 1995).

In experiment 2, the regression between temperature and the developmental rate was not statistically significant, probably due to few tested temperatures and low accuracy in setting days of oviposition. However, experiment 2 provided the supplementary data that indicated the adaptation of *P. kumamotoensis* to higher temperature compared with *P. penetrans*. Estimated duration of the *P. kumamotoensis* life cycle is shorter than that of *P. penetrans* at 30°C (15 days against 22.4 days (Mizukubo and Adachi, 1997)), while it is longer at 20°C (40 days against 38.5 days). Estimated base temperature for life cycle (14.3°C) was closer to tropical and subtropical species, e.g. *M. incognita* (10.1°C) and *M.*

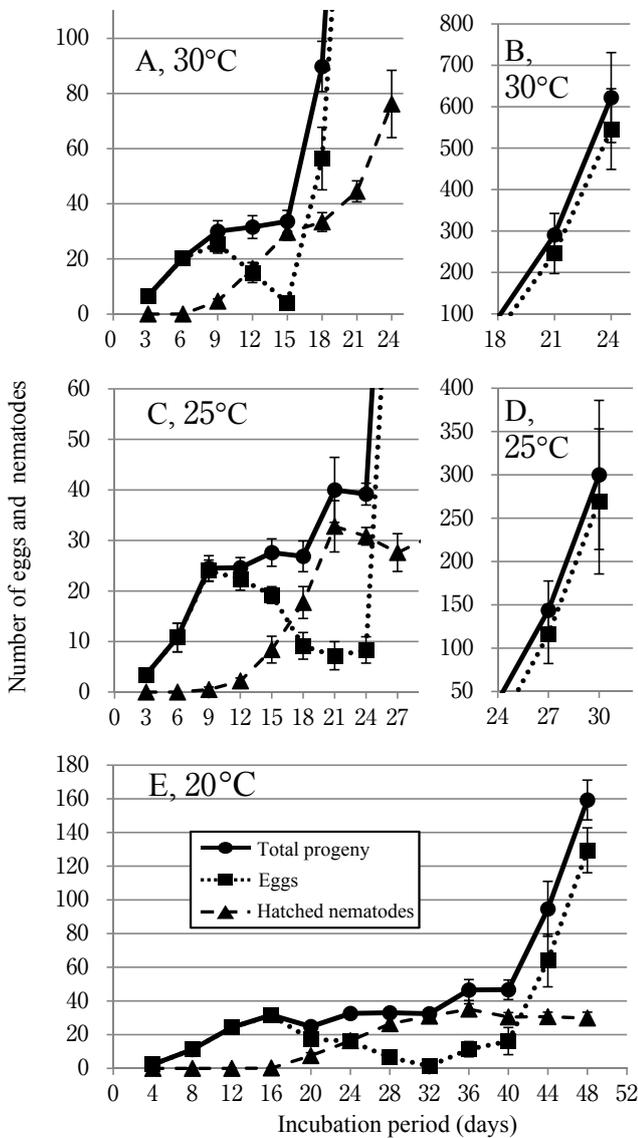


Fig. 2. Fluctuation in numbers of eggs, hatched nematodes and total progeny from a single female of *Pratylenchus kumamotoensis* inoculated in chrysanthemum roots. A and B, 30°C; C and D, 25°C; E, 20°C. Bars show standard error.

javanica (12.9°C) (Madulu and Trudgill, 1994; Ploeg and Maris, 1999), than to temperate *P. penetrans* (5.1°C).

This study showed that *P. kumamotoensis* is a chrysanthemum nematode pest adapted to higher temperatures than *P. penetrans*. This may cause geographical variation in the main causative species of chrysanthemum damage in Japan. It is also possible co-infested fields have a seasonal switch in the dominant species. Further data on their distribution and seasonal population fluctuation in fields are needed to evaluate the practical effect of nematode thermal characteristics on chrysanthemum nematode control.

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