HOST AND TEMPERATURE PREFERENCE, MALE OCCURRENCE AND MORFOMETRICS OFFUNGIVOROUS NEMATODE, *APHELENCHUS AVENAE* ISOLATES FROM JAPAN

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ABSTRACT

Host and temperature preference, male occurrence, and morfometrics of 39 isolates of *Apehelenchus avenae* were investigated. Of the 39 isolates, 33 were from 7 districts of Kyushu, 3 from Okinawa, and 2 from Ibaraki, and 1 from Fukushima prefecture of Hunshu. The host preferences were investigated on 4 species of fungi; *Botrytis cinerea, Rhizoctonia solani* AG-4, *Fusarium oxysporum* f. sp. *melonis,* and *Pytium aphanidermatum* growing on 1/5 strength PDA medium. The nematodes were incubated on respective fungus mat for 30 days at 25 and 30^{0}C. Host fungi and temperatures gave rise to various levels of reproduction and male occurrence. The isolates were devided into 5 groups based on their reproductivity on their host fungi at the 2 temperature regimes. Most of the isolates of *A. avenae* propagated themselves at 25^{0}C on *B. cinerea* and *R. solani* AG-4, whereas some isolates, especially from Okinawa and Kogoshima (southern parts of Kyushu) preferred 30^{0}C. the highest multiplication from the initial 10 females at 25^{0}C, 30 days after inoculation on *B. cinerea* was 60.850 given by Nagasaki isolate (NA3). The maximum multiplication 48.420 at 30^{0}C occurred on *R. solani* by Kagoshima isolate (KA3). The occurrence of males was very low in all isolates and it occurred only at 30^{0}C. De Man's value of body dimension were measured and showed no significant variations among the isolates. Besides, there were no relations between groupings by host preference and by body dimension.

Key words : *Aphelenchus avenae,* isolates, host preference, male occurrence, morphometrics, temperature preference.

INTRODUCTION

The fungivorous nematode, *Aphelenchus avenae* Bastian, 1865 is an ubiquitous species, living in most soils of the world (Hooper, 1974). At least 92 species of fungi under 52 genera, including both phytoparasitic and saprophytic species, have been recorded as food for *A. avenae* (Barnes *et al*., 1981; Townshend, 1964; Walker, 1984). Based on its feeding habit many attempts have been made using *A. avenae* as a biological control agent against different soil-borne fungal diseases. Either pot or glasshouse experiments, the verulence of *A. avenae* against many soil-borne fungal pathogens of economic inportance (Barker, 1964) and the availability of simple and inexpensive methods for its mass-production using industrial/agricultural by-products (Choi, 1994) make this nematode a promising attractive candidate as a commercial biological control agent (Evans, 1970).

However, many problems remain to be overcome for the mass production or commercial use of this beneficial nematode. For example, reproduction of 5 isolates of *A. avenae* collected from Japan showed significant differences in freferences to host fungi, preferable temperature, and dextrose content of the PDA medium (Choi, 1994).

Out of 4 Australian isolates, 3 reached the fastest reproduction at 30^{0}C, and the others did at 25^{0}C (Evan & Fisher, 1970b). The occurrence of males in some Australian isolates ranged from very rare to very common (Evan & Fisher, 1970a). The egg deposition in both parthenogenetic and amphimictic females were greatly related to male occurrence (Fisher, 1972). In Japan, a detailed description of the characteristics of *A. avenae* isolates from Kyushu and other parts of Japan is nematodes pertinent to the control of target soil phytopathogenic fungi. This paper reports the host and temperature preferences, male occurrence, and morphometrics of 39 isolates of *A. avenae* from Kyushu and some other parts of Japan.

MATERIALS AND METHODS

Thirty nine isolates of *Aphelenchus avenae* used in the present studies, were collected mainly from Kyushu and some other parts of Japan (Table 1). Of the 39, 33 were from 7 prefectures of Kyushu and remaining 6 were : from Okinawa, 2 from Ibaraki, and 1 from Fukushima. Each isolate was extracted from the soil by *Baermann* funnel, surface sterilized with streptomycin sulphate (1.000µg/ml) for 30 min, then rinsed 5 times with sterilized distilled water. A singel female adult from each isolate was introduced onto fungal mat of *Botrytis cinerea* growing in a petri dish (9 cm-dia) with 1/5 strength PDA medium and incubated at 25^{0}C. The population developed from this single female for 1 month was used as stock culture for the following experiments.

Host and temperature preference : Four fungal species namely: *B. cinerea, Fusarium oxysporum* f. sp. *melonis, Rhizoctonia solani* AG-4, and *Pythium aphanidermatum* were used as the host fungi for *A. avenae*. Except *B. cinerea,* which was originally cultivated in the laboratory of Plant Pathology, Saga University, the other three fungi were introduced from Kyushu National Agriculture Experiment Station, Kumamoto. Ten female adults from each isolate were hand picked under stereo microscope and inoculated to the above fungi growing in the same sized petri dish containing 1/5 strength PDA medium. The petri dishes were sealed with parafilm and incubated at 25 and 30^{0}C with 5 replicates.

Thirty days after inoculation, the nematode populations developed on each fungus at respective temperature were harvested by *Baermann* funnel and recorded . The occurrence of males in each isolate at 30^{0}C was examined by observing 200 randomly collected adults under compound microscope (X 200). Thes nematodes were gently heat-killed by immersing in 55^{0}C hot weter for 10 min and fixed in cold TAF prior to observation. All isolates of *A. avenae* failed to multiply on the fungus *P. aphanidermatum* at 30^{0}C. Therefore, male frequency was recorded from the remaining 3 host fungi; *B. cinerea, R. solani* AG-4, and *F. oxysporum* f. sp. *melonis.*

Morphometrics : Female adults of *A. avenae* from 39 isolates cultured on *B. cinerea* at 25^{0}C were employed for the measurement of body dimensions. Twenty adult females were killed by gentle heat, fixed in TAF, and transferred to ethanol glycerin (modified after Seinhorst, 1959). De Man's values for the various morphological categories were adopted for the measurement of dimensions. The data were statistically analyzed by Tukey's Studentized Range-Test ($p = 0.05$)

Tabel 1. Isolate numbers, code numbers, origins and associated vegetation of 39 isolates of *Aphelenchus avenae* from Kyushu and some other parts of Japan.

No	Code no.	Origins	Associated vegetation
1	FU1	Hiwaki, Fukuoka	Persimmon tree
2	FU2	Hiwaki, Fukuoka	Broccoli
3	FU3	Dazaifu, Fukuoka	Soybean
4	FU4	Munakata, Fukuoka	Soybean
5	FU5	Kurume, Fukuoka	Spinach
6	NA1	Sasebo, Nagasaki	Broccoli
7	NA2	Hukue, Nagasaki	Sweet potato
8	NA3	Kishuku, Nagasaki	Taro
9	SA1	Kinryu, Saga	Buedock
10	SA2	Kubomizu, saga	Sweet potato
11	SA3	Takeo, Saga	Spinach
12	SA4	Hamatama, Saga	Welsh onion
13	SA5	Ogi, Saga	Mandarin orange
14	SA6	Nakayama, Saga	Lettuce
15	SA7	Hizen, Saga	Pea
16	OH1	USA, Ohita	Strawberry
17	OH2	USA, Ohita	Egg plant
18	OH3	USA, Ohita	Scallion
19	OH4	USA, Ohita	Water melon
20	OH5	USA, Ohita	Water melon
21	KU1	Nashigoushi, Kumamoto	Buck wheat
22	KU2	Nashigoushi, Kumamoto	Upland rice
23	KU3	Takamori, Kumamoto	Cabbage
24	MI1	Ebino, Miyazaki	Common marigold
25	MI2	Shintomi, Miyazaki	Pea
26	MI3	Miyakonojo, miyazaki	Welsh onion
27	MI4	Miyakonojo, miyazaki	Japanese radish
28	KA1	Kushira, Kagoshima	Sweet potato
29	KA2	Kushira, Kagoshima	Japanese radish
30	KA3	Kushira, Kagoshima	Soybean
31	KA4	Izumi, Kagoshima	Cauliflower
32	KA5	Ohsumi, Kagoshima	Carrot
33	KA6	Kushikino, Kagoshima	Welsh onion
34	OK1	Rukyu, Okinawa	Sugar cane
35	OK2	Gushiken, Okinawa	Taro
36	OK3	Gushiken, Okinawa	Sweet potato
37	IB1	Tsukuba, Ibaraki	Pea
38	IB2	Tsukuba, Ibaraki	Tea
39	FKSM	Fukushima, Fukushima	Elephant foot

RESULTS

Host and temperature preferences : The rates of multiplication of 39 isolates of *A. avenae* 30 days after inoculation from the initial 10 females on the 4 host fungi were shown in Table 2. Among the 4 fungi tested as host of *A. avenae* at the 2 levels of temperature, the rate of multiplication of different isolate on different hosts varied depending on the incubation temperatures. For example, the rates on the hosts *B. cinerea* and *F. oxysporum* were always higher at 25^{0}C than at 30^{0}C on the respective fungus, though overall reproduction of every isolate at any given temperature was higher on *B. cinerea* then on *F. oxysporum* (Table 2). In the contrast, the nematode multiplication on *R. solani* AG-4 did not follow the same tendency. Out of 39 isolates, the populations developed from 22 isolates at 30^{0}C outnumbered those developed from the same isolate at 25^{0}C. The remaining 18 isolates multiplied equally at both temperatures or better at 25^{0}C than at 30^{0}C.

The fungus *P. aphanidermatum* was the poorest host for propagation of *A. avenae* at 30^{0}C. All isolates failed to multiply at this temperature. Population development at 25^{0}C on this fungus was very poor, ranging from 100-2.200 (Table 2), and a few isolates established higher populations.

The highest (60.850) and the second highest (58.450) multiplication of *A. avenae* at 25^{0}C occurred on the fungus B. cinerea for the isolates NA3 and OH1, respectively. The maximum multiplication at 30^{0}C occurred on the fungus *R. solani* AG-4 by isolate KA3 (48.430) followed by KA6 (43.500) both of them are located in southern Kyushu. The maximum population development on F. oxysporum at 25^{0}C was by isolate OH2 (23.600) followed by isolate MI2 (23.074).

Male occurrence: Male frequen- cy in different isolates of *A. avenae* on the 3 fungi tested was generally very low, ranging from 0 to 3% (Table 3). The males appeared only at high incubation temperature (30^{0}C). Out of 39 isolates 11, 10, and 5 isolates produced males ranging 0,5-1,5%, 0,5-3%, and 0,5-2%, on the host fungi *B. cinerea*, *R. solani* AG-4, and *F. oxysporum*, respectively. The highest male ratio (3%) was recorded on the host *R. solani* by isolate MI2, followed by 2% by isolate SA5, SA6, FU3, and KA5. On *B. cinerea* the maximum male ratios were 1,5% by the 2 isolates OH2 and KU3. Only isolate OH4 produced males on *F. oxysporum* by 2%.

Morphometrics: The morphome- trics of 39 isolates of *A. avenae* from Kyushu and other parts of Japan showed statistically no significant variation for "L", "a", "b", "c", "c' ", and "V" (Table 4). The longest body length 0,86 mm was exhibited by isolates OK3 and IB2, whereas the shortest body length 0,73 mm was exhibited by isolates FU1, OH3 and FKSM. The difference in body dimensions for each isolate did not any corelations with host or temperature preference.

Based on the host and temperatu- re preference, 39 isolates of *A. avenae* from Kyushu and some other parts of Japan were divided into 5 groups (Table 5). In the group I, 4 isolates multiplied best on *B. cinerea* at 25^{0}C. In the group II, 4 isolates reproduced both *B. cinerea* and *R. solani* at 25^{0}C. In the group III, 15 isolates maximized their populations best on *B. cinerea* and on *R. solani* at both 25 and 30^{0}C, whereas 13 isolates of group IV performed their reproduction well on the 3 host fungi; *B. cinerea*, *R. solani*, and *F. oxysporum* at 25^{0}C. The last group V, contained 3 isolates that reproduced well on the 4 host fungi tested at both temperatures.

Tabel 2. Reproduction (x 100) of 39 isolates of *Aphelenchus avenae* from Kyushu and some other parts of Japan on 4 fungi growing on PDA medium incubated at 25 and 30°C. The reproduction was examined 30 days after inoculation with females*

Isolate	B. c**		R. s**		F. o**		P.a**
	25°C	30°C	25°C	30°C	25°C	30°C	25°C
FU1	351,0	125,0	206,0	173,3	114,3	35,0	3,3
FU2	285,0	189,7	218,3	312,0	86,8	34,0	4,1
FU3	424,0	254,0	260,0	150,8	137,7	48,7	15,5
FU4	271,0	147,5	223,0	134,0	113,5	95,9	7,5
FU5	314,0	112,5	228,5	158,7	115,8	34,5	12,0
NA1	320,0	131,8	253,7	271,5	80,0	25,5	5,2
NA2	355,0	107,0	256,7	290,0	213,7	135,5	6,2
NA3	608,5	134,0	376,5	518,1	90,4	26,7	3,5
SA1	482,0	152,5	122,0	218,5	149,5	22,2	12,5
SA2	377,5	171,7	254,2	278,2	225,5	51,5	12,1
SA3	584,5	78,5	197,7	234,0	128,5	30,7	8,5
SA4	553,0	57,0	201,2	306,4	130,9	17,5	3,2
SA5	431,3	64,0	325,7	180,0	99,0	19,5	11,2
SA6	460,8	39,6	302,5	279,9	85,5	26,5	4,5
SA7	331,3	125,0	313,7	283,7	168,0	36,1	3,2
OH1	536,8	151,0	224,8	152,2	163,5	26,7	2,9
OH2	508,8	74,0	233,0	84,7	236,5	31,5	3,0
OH3	294,8	94,5	231,0	100,0	186,7	105,5	22,6
OH4	342,0	65,5	214,2	89,5	146,5	20,0	2,2
OH5	296,8	70,5	230,3	66,5	90,0	49,2	1,9
KU1	294,0	123,6	297,5	129,5	123,5	34,9	2,8
KU2	351,0	196,5	353,5	250,0	73,2	34,3	1,0
KU3	240,0	144,5	246,0	146,5	111,5	24,5	3,4
MI1	307,0	56,0	205,0	146,2	70,7	20,7	3,7
MI2	451,0	154,5	341,0	149,5	230,7	100,0	12,1
MI3	332,0	86,7	116,0	117,7	78,5	17,6	1,8
MI4	402,0	121,5	244,5	93,5	74,5	19,7	4,2
KA1	436,0	255,0	202,9	296,5	113,3	26,5	1,0
KA2	412,1	295,0	194,5	366,5	10,5	30,0	3,2
KA3	331,0	79,3	257,6	484,2	138,0	21,0	4,7
KA4	197,0	73,8	228,5	274,0	226,3	116,0	2,5
KA5	406,5	114,0	244,5	213,5	87,5	37,0	4,0
KA6	414,4	121,6	220,5	435,0	66,0	70,0	4,5
OK1	330,0	152,0	384,0	373,0	197,0	45,0	11,0
OK2	188,0	72,0	384,0	292,0	223,0	76,0	1,6
OK3	437,0	86,0	427,0	342,0	182,0	126,0	7,5
IB1	425,0	106,0	426,0	382,0	79,0	64,0	3,0
IB2	378,0	81,0	362,0	397,0	110,0	53,0	4,0
FKSM	237,0	63,0	176,0	79,0	70,0	65,0	6,0

* = Data are means of five replicates

** B. c = *Botrytis cinerea*, R. s = *Rhizoctonia solani* AG-4, F. o = *Fusarium oxysporum* f. sp. *melonis*, P. a = *Pythium aphanidermatum*

Table 3. Male frequency (%) of 39 isolates of *Aphelenchus avenae* from Kyushu and some other parts of Japan on the host fungi *Botrytis cinerea* (B. c), *Rhizoctonia solani* AG-4 (R. s), and *Fusarium oxysporum* f. sp. *melonis* (F. o) growing on 1/5 strength PDA medium incubated at 30^{0}C*.

Isolates	B. c	R. s	F. o
FU1	0,5	1,0	0
FU2	0	0	0
FU3	0	0	0
FU4	0	0	0
FU5	0	0	0,5
NA1	0	0	0
NA2	0	0	0
NA3	0	0,5	0
SA1	0	0	0
SA2	O,50	0	0
SA3	0	0	0
SA4	0	0	0
SA5	0	0	0
SA6	0	2,0	0,5
SA7	0	2,0	0
OH1	0,5	1,0	1,0
OH2	1,5	0	0
OH3	0	0	0
OH4	0	0	2,0
OH5	0	0	0
KU1	0,5	0	0
KU2	0	0	0
KU3	1,5	0	0
MI1	0	1,0	0
MI2	0	3,0	0
MI3	0	0	0
MI4	0	0	0
KA1	0	0	0
KA2	0	0	0
KA3	0	0	0
KA4	0,5	1,0	0
KA5	0	2,0	0
KA6	0,5	0	0
OK1	0	0	0
OK2	0,5	0	0
OK3	0	0	0
IB1	0	0	2,0
IB2	0	0	0
FKSM	1,0	0	0

* Data were obtained by observing 200 randomly selected adults under microscope (X 200)

Table 4. Morphometrics of 39 isolates of *Aphelenchus avenae* from Kyushu and some other parts of Japan*.

Isolates	L (mm)	a	b	c	c'	V (%)
FU1	0,73	26,20	9,20	29,30	1,47	75,30
FU2	0,74	27,10	8,90	30,70	1,50	72,93
FU3	0,78	26,67	8,60	24,44	1,50	75,26
FU4	0,77	26,04	9,18	24,19	1,63	75,14
FU5	0,74	26,37	8,71	25,20	1,63	76,04
NA1	0,81	25,61	9,25	28,57	1,40	74,35
NA2	0,85	26,85	9,17	29,46	1,62	77,09
NA3	0,75	29,56	8,57	26,77	1,68	76,02
SA1	0,81	22,17	8,77	26,54	1,41	78,55
SA2	0,85	21,26	8,72	22,00	1,38	80,44
SA3	0,83	24,55	8,36	22,79	1,36	74,35
SA4	0,84	21,45	8,92	21,75	1,54	74,75
SA5	0,82	22,86	9,39	26,13	1,44	76,07
SA6	0,80	24,74	9,82	26,34	1,46	71,89
SA7	0,80	23,05	8,50	22,38	1,46	75,68
OH1	0,74	23,55	8,76	28,00	1,55	76,62
OH2	0,73	26,10	9,00	27,10	1,60	77,25
OH3	0,84	27,50	8,60	27,50	1,40	74,90
OH4	0,85	26,80	8,40	28,30	1,40	76,11
OH5	0,84	24,70	9,53	25,99	1,54	75,80
KU1	0,85	23,57	9,48	24,34	1,32	75,43
KU2	0,79	27,79	9,11	23,72	1,69	78,10
KU3	0,83	26,20	9,91	31,07	1,65	78,51
MI1	0,86	26,10	9,27	30,98	1,66	75,43
MI2	0,81	28,05	9,34	29,68	1,48	76,65
MI3	0,85	29,30	10,29	34,63	1,38	76,60
MI4	0,81	27,60	9,68	32,13	1,44	77,74
KA1	0,85	28,74	9,87	27,25	1,36	71,15
KA2	0,79	26,50	9,00	26,50	1,59	75,87
KA3	0,75	27,80	8,80	28,40	1,68	76,09
KA4	0,84	27,59	10,01	26,24	1,55	76,56
KA5	0,80	27,10	9,30	25,78	1,55	75,45
KA6	0,76	24,12	8,85	24,37	1,61	75,91
OK1	0,79	29,67	9,10	30,35	1,83	78,50
OK2	0,83	31,49	9,78	28,64	1,77	79,29
OK3	0,86	32,44	10,23	30,51	1,71	76,70
IB1	0,80	24,68	9,18	24,33	1,58	75,39
IB2	0,86	23,92	9,60	25,78	1,48	76,06
FKSM	0,73	32,21	8,80	24,54	1,92	78,50

*n = 20 female adults.
L = total body length in mm
a = body length/greatest body width
b = body length/distance from the anterior end to base of median oesophageal bulb.
c = body length/tail length
c' = tail length/body length at anus
V = (distance of vulva from anterior end) x 100/body length.
Values followed by the same letters are not significantly different by Turkey's Studentized Range Test (p = 0,05).

Table 5. Grouping of 39 isolates of *Aphelenchus avenae* from Kyushu and some other parts of Japan based on host and temperature preference.

Group	Host fungus/Fungi*	Temperatures (^{0}C)	Isolates
I	B	25	OH1, 2, 4, & MI3
II	BR	25	SA5 ; MI1, 4 & FKSM
III	BR	25/30	FU2 ; NA1, 3 ; SA6 ; KA1, 2, 3, 4, 5, 6 ; OK1, 2, 3 ; IB1 & 2
IV	BRF	25	FU1, 4, 5 ; NA2 ; SA1, 2, 3, 4, 7 ; OH5 ; KU1, 2 & 3.
V	BRFP	25/30	FU3 ; OH3 & MI2.

*B = *Botrytis cinerea*, R = *Rhizoctonia solani* AG-4, F = *Fusarium oxysporum* f. sp. *melonis*, P = *Pithyum aphanidermatum*

DISCUSSION

Several investigations have been made on ecological differences among isolates of *A. avenae*, especially on the temperature optimal and propagation rates (Hansen *et. al.* 1973 ; Monoson, 1971 ; Pill & Taylor 1967). Thirty nine isolates from Kyushu and other parts of Japan had different preference for host and/or temperature. The difference in propagation among isolates may be attributed to the potential fecundity of nematode or suitability of the host fungus (Choi & Ishibashi, 1989 ; Evans, 1970)

Tawnshend (1964) reported that the best food of *A. avenae* was *B. cinerea* on PDA medium. Most isolates used in the present investigation propagated themselves almost to the same level on *B. cinerea* at 25^{0}C. However, some isolates preferred *R. solani* as food, when the temperature was raised to 30^{0}C. All isolates fed and multiplied on *F. oxysporum*, but the retes were smaller than on *B. cinerea* or *R. solani*. Very few isolates, e.g., isolates FU3, OH3, and MI2, reproduced poorly on *P. aphanidermatum* only at 25^{0}C. The difference in the number of nematodes propagated on various host fungi may be due to the difference capacity of the fungus to support the growth and reproduction (Fisher, 1970). Individual fungi have considerable variations in their chemical compositions, and qualitatively (Norton, 1978).

For *A. avenae*, there was a common tendency for male to appear at high temperature (Buecher & Hanse, 1974), or males were very rare or absent throughout experimental period when the temperature was 25^{0}C (Choi & Ishibashi, 1989). In the present investigation, too, no males did appear at 25^{0}C, and at 30^{0}C males appearance ranged from 0,5% in some isolates to maximum 3% on *R. solani* in MI2.

The present investigation we employed only descriptve taxonomy using different morphometrics as the creteria to differentiate the *A. avenae* isolates from Japan. In the descriptive systimatics the populations are placed under the same taxonomic group with identical morphometrics. Variations in the nematode morphometrics should have been caused by environmental condition. Considering the fact that, different populations of the same nematode recovered from different localities showed some variations in the morphometrics (Norton, 1978) and the morphometrics of *A. avenae* populations also varied considerably and to some extent morphology and *A. avenae* may represent a species complex (Ishibashi *et. al.*, 1997). Excepting the difference of 130 μm between the maximum and minimum in body length, the 39 isolates of *A. avenae* used showed no significant variations in any morphometrics. This result may indicate that all isolates used in the present investigation are morphometrically *A. avenae*. In additian, these morphological differences seemed to have no relation with their host preference. Further studies are under way using melecular and biochemical techniques to differentiate these isolates, and to compare and correlate with the results of present investigations.

Aphelenchus avenae was applied for biological control of *Rhizoctonia* stem canker of potato at such low temperatures as 10-15^{0}C (Lootsma & Scholte, 1997a) and at a wide range of soil moisture contents (Lootsma & Scholte, 1997b), and of Cody alfalfa root rot caused by *R. solani* and *F. solani* at such high temperatures as 23-33^{0}C (Barnes *et. al.*, 1981). The results suggested that this nematode is able to acclimatize to every wide range of local temperature regimes and other soil coditians. Based on the growth and reproduction of respective isolate on 4 host fungi and 2 levels of temperature, the 39 isolates of *A. avenae* from Kyushu and other areas of Japan were sorted to 5 categories. The isolates originated from the warmer areas like Okinawa and Kagoshima well performed at both 25 and 30^{0}C. comparatively most isolates from other relatively cooler regions performed only at 25^{0}C. The present investigation demonstrated that the Japanese isolates of *A. avenae* also adapted locally. Most of them preferred 25^{0}C but some isolates preferred both 25 and 30^{0}C equally. The letter can be of promising biological control agent in such warm areas as tropical and subtropical ones.

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