Aphelenchus avenae（アフェレンクス科）とFilenchus misellus（ティレンクス科）にRhizoctonia solani菌7系統を餌として与えた場合の増殖性

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<th>誌名</th>
<th>Nematological research</th>
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<tr>
<td>掲載ページ</td>
<td>p. 53-55</td>
</tr>
<tr>
<td>発行年月</td>
<td>2010年12月</td>
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農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Propagation of *Aphelenchus avenae* Bastian (Aphelenchidae) and *Filenchus misellus* Andrássy (Tylenchidae) on seven fungal isolates of *Rhizoctonia solani* as food source

Hiroaki Okada 1, Hiroki Harada 2 and Mana Nozaki 1

The fungivorous nematode *Aphelenchus avenae* Bastian (Aa) is purported to be a promising biocontrol agent against soil-borne phytoparasitic fungi because of its ability to reduce the amount of fungal hyphae. Actually, the nematode effectively controlled *Rhizoctonia* wilt by its application into the soil (Barker, 1964; Ishibishi, 2005; Okada, 2006). There are significant problems with the use of nematodes, however, in that their propagation rates differ among the various nematode isolates and among the fungal isolates used as food source (Mankau and Mankau, 1962; Bonnel and Camporota, 1989). When we consider the practice of biocontrol, the agent organism should be an indigenous rather than an alien species, so as to minimize the impact on the native ecosystem. Thus, the indigenous isolate of Aa should be examined for its propagation properties before it is used in a country or a region.

For the Japanese Aa isolates, the differences in the propagation rates among isolates have already been examined (Choi and Ishibashi, 1989; Ali et al., 1999); however, differences in the rates caused by the fungal food isolates have not yet been examined.

*Rhizoctonia solani* (Rs) isolates themselves are a useful food source for nematode cultures because they lack spores and have wide hyphae. Owing to these properties, we can obtain nematodes without the need for sterile treatment using harmful chemicals, if we use an appropriate filter paper to extract nematodes from a monocytic culture (Chen and Ferris, 1999; Okada et al., 2005).

We have reported that some nematode species of the genus *Filenchus*, a common group of the family Tylenchidae, have a potential to feed on fungi including Rs (Okada et al., 2002; Okada and Kadota, 2003; Okada et al., 2005). This is an important finding for the ecological study of soil nematodes (Ferris and Bongers, 2009) and for biocontrol of soil-borne phytoparasitic fungi. The species of *Filenchus* are also good models for studies on ecological interactions with nematophagous fungi (Okada and Kadota, 2003). Their propagation on Rs isolates, however, has not yet been measured in detail.

This paper reports the compatibility of Aa and *Filenchus misellus* Andrássy (Fm) as biocontrol agents and the importance of host fungal isolates of Rs on the propagation of the two nematode species.

**MATERIALS AND METHODS**

Seven Rs isolates were tested (Table 1). The two nematode species were from our laboratory stock cultures (Okada and Kadota, 2003). To measure nematode propagation, fungal colonies were prepared in Petri-dishes as described in Okada et al. (2005). *Filenchus misellus* and Aa were maintained on R19 colonies on PDA to be used as inocula before experiments. The nematodes were extracted from PDA under aseptic conditions, and 30 individuals of either Aa or Fm were introduced to each Petri-dish. There were four replicates for each nematode species-fungal isolate combination. The dishes were incubated at 25°C in the dark for 40 days for nematode propagation. The nematodes were then extracted from each dish by the Baermann funnel method (Okada et al., 2005) and counted. The experiment was conducted twice: first from May 29th to July 8th and second from Sept. 25th to Nov. 4th, 2002. A generalized linear model (glm) approach was taken presuming a negative binomial model for statistical analysis. First, the effects of nematode species and fungal isolates were examined by the stepAIC function of a statistical package, "R" (R Development Core Team, 2005), with the following formula,

\[
\text{glm.nb (nematode count~x+y+x:y),}
\]

where x, y, x:y were nematode species, fungal isolate, and interaction of x and y, respectively. Then, to examine

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Table 1. *Rhizoctonia solani* isolates used in the study.

<table>
<thead>
<tr>
<th>ID</th>
<th>Original ID</th>
<th>Mating type</th>
<th>Source crop</th>
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<tr>
<td>R03</td>
<td>MAFF 35003</td>
<td>AG-1</td>
<td>Rice (Oryza sativa)</td>
</tr>
<tr>
<td>R19</td>
<td>MAFF 350219</td>
<td>AG-1</td>
<td>Rice (Oryza sativa)</td>
</tr>
<tr>
<td>S52</td>
<td>MAFF 305252</td>
<td>AG-4</td>
<td>Soybean (Glycine max)</td>
</tr>
<tr>
<td>S61</td>
<td>MAFF 237261</td>
<td>AG-3-3</td>
<td>Soybean (Glycine max)</td>
</tr>
<tr>
<td>C24</td>
<td>MAFF 237424</td>
<td></td>
<td>Cabbage (Brassica oleracea)</td>
</tr>
<tr>
<td>C37</td>
<td>MAFF 305237</td>
<td>AG-2-1</td>
<td>Cabbage (Brassica oleracea)</td>
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<td>RsT 1</td>
<td>-</td>
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</tr>
</tbody>
</table>

1 From the culture collection of the former Laboratory of Crop Protection, National Agricultural Research Center for Tohoku region, Fukushima city, Fukushima, Japan.
2 Provided by the genebank of National Institute of Agrobiological Sciences, Tsukuba city, Ibaraki, Japan.
effects of the fungal isolate on each nematode species, multiple comparisons were made on the basis of Akaike's Information Criterion (AIC) such that
\[
glm.nb(\text{nematode count}) = \text{count} - z,
\]
where \(z\) is a possible grouping of fungal isolates that could minimize AIC (Kasuya and Kubo, 2004).

RESULTS AND DISCUSSION

The results of the first and second experiments were similar for each of Aa and Fm in terms of nematode counts and fungal species relationships. The minor differences in the second experiment were 1) nematode counts of Aa and Fm were generally smaller for uncertain reasons, and 2) the Aa count on C24 was especially smaller probably owing to irregular and inconsistent fungal growth. Because of the overall similarity, however, we report here only the results of the first experiment. Each of the nematode species, fungal isolates, and their interactions affected the nematode counts according to the stepAIC function. The mean count of Fm (maximum: 500/dish) was much smaller than that of Aa (minimum: 8300). For each nematode species, the counts differed among the fungal isolates (Fig.1). According to the glm.nb model, the numbers of the fungal isolate groupings were three for Aa and four for Fm. Aa counts were greater on S52, C24, and RsT, while smaller on R03, R19, and C37. Fm counts were greater on R03, S52, and C24, while smaller on S61.

It had been reported that propagation of French and African isolates of Aa greatly differed, from one to 20 times the inoculated number, depending on the combinations of the nematode and the Rs isolates (Bonnel and Camporota, 1989). For the Japanese isolates of Aa, however, the effects of nematode isolates on Aa propagation had already been examined on a single Rs isolate (Choi and Ishibashi, 1989), and the effects of Rs isolates had not yet been tested. Our study showed that generally Aa propagated well across the seven Rs isolates tested, although the degree of propagation differed among them (the nematode counts ranged from 275 to 1449 times the inoculated number). No clear trend was observed in the relationship between the nematode propagation and the source crops of the fungal isolates, although the propagation tended to be less on the Rs isolates from rice. The Aa isolate in our study may be used as a control against every Rs isolate tested, especially S52 and C24; however, other Japanese Aa isolates might provide different results. We should examine in advance how fungal isolates affect the nematode propagation, when we consider the use of an Aa isolate to control for a given fungal species.

The propagation of Fm was much smaller than that of Aa across the fungal isolates tested (the nematode counts were 0.02 to 16.5 times the inoculated number), thus it could be difficult to achieve biological control against the fungi with this nematode. Unsuccessful propagation of nematode species of the family Tylenchidae on Rhizoctonia had already been reported elsewhere (Ferris et al., 1996). Nevertheless, our study suggested that Fm could propagate to a certain degree, if R03, S52, C24, or RsT were selected as the food source. By using these Rs isolates to maintain the stock nematode cultures, Fm can be used as a model nematode, which can feed on and reproduce on such nematophagus fungi as Pleurotus spp. (Okada et al., 2003). Further studies may reveal interesting relationships between the nematode and the predacious fungi.

LITERATURE CITED


Bonnel, L. and Camporota, P. (1989) Influence of the strain of Rhizoctonia solani (Kühn, 1858) on multiplication of the mycophagous nematode, Aphelenchus avenae (Bastian, 1865). Nematologica 35 105-114. (in French with an English summary)


Received: July 23, 2010
ヤガ類幼虫に対する防除能力をスクリーニングされた日本産昆虫病原性線虫（Steinernema属）の病原性に温度がおよぼす影響
吉田隆雄 .................................................. 27

生物防除に供しうる土着のSteinernema属昆虫病原性線虫を選定するために、ヤガ類幼虫に対する病原性を調査した。最初に、土着種10種17アイソレートの病原性をニセタマヤガ中幼虫を用いて比較した結果、S.feltiaeとS.litoraleが選定された。次いで、前者2、後者8アイソレートの病原性をカブラヤガ中幼虫を使って比較した結果、S.feltiaeが最も病原性が高く、カブラヤガと北条城アイソレートがそれぞれ選定された。25℃以下では高い病原性を示したが、30℃では低下した。そこで、S.abbasi西表島アイソレートを加えて、3アイソレートのカブラヤガ、ハモンソウロト老齢幼虫に対する病原性を導入種S.carpocapsae Allと比較した。S.litorale北条城は7℃ではの結果が示すように高い病原性を示し、S.carpocapsae Allと比較して7℃で10℃でカブラヤガ、7℃でハモンソウロトに対し高い病原性を示した。S.abbasi西表島はS.carpocapsae Allと比較して30℃、35℃でカブラヤガ、35℃でハモンソウロトに対し高い病原性を示した。

土壌固めめ構法あるいはボールミル法により調整された土壌中のジャガイモ細胞質センチュウの直接定量のための検量線の比較
後藤圭太・佐藤恵利華・木崎剛明・豊田萌子・杉村貴子 .................................................. 41

土壌固めめとリアルタイムPCRからなる定量化方法を、土壌中のジャガイモ細胞質センチュウ（PCN）の定量に適用した。また、線虫線虫の破壊作と一画に土壌サンプルの入手の効率、2つの前処理法（ボールミルと土壌固めめ）を比較した。2種類のクレオキスを用いてこれらの実験を行った。いずれの土壌、いずれの前処理法でも、土壌20g当り5個のPCN2個幼虫（2J）を検出でき、Cr値と2Jの添加数との関係には、r = 0.8697の有意な相関が認められた。Cr値と土壌中の2J密度との関係を示す検量線は、2つの土壌では2つの前処理間でほぼ同じであったが、1つの土壌ではCr値がボールミル法で高くなり、2個低い値を示したところ、DNA抽出効率はボールミル法の方が良いであると推察された。ボールミル法で得られた検量線は土壌ではほぼ同一であったが、検量線法では異なっていたことから、ボールミル法の方が線虫の直接定量には相応しいと考えられた。

ベニツチカメムシに自然状態で寄生するCaenorhabditis japonica耐久型幼虫の生存性
田中龍聖・奥村淳子・吉野豊司 .............................. 47

Caenorhabditis japonica Kiontke, Hirronaka and Sudhausはベニツチカメムシに寄生する細菌性食性線虫である。休止状態のC.japonica耐久型幼虫は主にカメムシ成虫体表面から1年を通じて検出されることが考えられるが、カメムシ上で長期生存することが考えられるが、生活期は明らかでない。形態的特徴をみるためクライオ走査電子顕微鏡観察をおこなったところ、カメムシ上の線虫は緩やかな観水状態であったが、無菌生存性を有するイネシゲカメムシの下における観察はみられなかった。野外から採取したカメムシを実験室内でいくつかの湿度条件下におき、3か月保存後に観察し、カメムシ上の耐久型幼虫の生存を調べた。その結果、湿度100%においてはカメムシ上にほとんどの線虫は残っておらず、湿度97%においては1頭のカメムシあたり19頭の線虫が検出され、その生存率は33%であった。一方、木箱の箱で維持したカメムシではカメムシの生存率は100%となり、1頭のカメムシあたり67頭の線虫が検出され、その生存率は55%であった。以上の結果より、C.japonica耐久型幼虫はベニツチカメムシ上で緩やかに乾燥した休止状態になり、数か月間生存可能であることが明らかになった。

 Aphelenchus avenae (アフェレンクス科)とFilenchus misellus (ティレンクス科)にRhizoctonia solani菌種7系統を饵として与えた場合の増殖性
岡田浩明・原田啓基・野崎真奈 .................................. 60

アフェレンクス科の Aphelenchus avenae (Aa) とティレンクス科の Filenchus misellus (Fm) について、土壌伝染性植物病原性真菌Rhizoctonia solani (Rsa)の7つの系統を飼養させた場合の増殖性を調べた。いずれか1つのRsa系統の菌糸が生育したジャガイモ葉柄部にAaとFmのいずれか1種を30匹移植し、25℃で40日間増殖させた後、ペルマン漏斗で鏡下で菌糸を分離し、個体数を調べた。負の二項分布を仮定した統計モデルにより検討したところ、線虫種、Rsa系統及び両者の交互作用のいずれもが線虫個体数に影響したと考えられた。全般にAaでは個体数がFmより著しく多く、供試したRsa系統のうち、特にディズとキャベツから採取された系統で強く増殖した。一方Fmは、ほとんど増殖しなかったRsa系統があったが、イネ、ダイズ、キャベツに由来する系
統ではある程度の増殖が認められた。