Molecular Strategies to Control the Plant-Parasitic Nematodes *Meloidogyne chitwoodi* and *Pratylenchus penetrans*

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Introduction

Root-knot (*Meloidogyne* spp.) and root-lesion nematodes (*Pratylenchus* spp.) are biotrophic endoparasites that invade roots and tubers of host plants. They parasitize a wide variety of plant species and cause significant yield losses worldwide (Castillo and Vovlas 2007, Perry et al. 2009). In the Pacific Northwest, *M. chitwoodi* and *P. penetrans* are serious pathogens in potato. *M. chitwoodi* generally does not lead to dramatic quantitative yield losses in potato, but it causes quality defects on the tuber surface that can render entire shipments unmarketable. This species is a quarantine pathogen and hinders trade with key export markets. *P. penetrans* is a widespread pathogen in potatoes east of the Rocky Mountains, where it can lead to dramatic yield losses. *P. penetrans* is an emerging problem for potato production in the Pacific Northwest. Recently, this nematode has been found in potato fields in the Columbia Basin, where it caused yield declines of 4 tons/acre (Ingham et al. 2005). *P. penetrans* is part of the potato early dying disease complex, in which it interacts with the fungal pathogen *Verticillium dahliae* (Rowe and Powelson 2002). It is likely that *P. penetrans* will continue to spread in potato producing regions in the Pacific Northwest, which might lead to a significant increase in production costs. Both *M. chitwoodi* and *P. penetrans* have wide host ranges and are difficult to control with crop rotation. At present, there are no potato cultivars that are resistant to these nematodes. Current control strategies rely on synthetic nematicides, but increasing limitations of chemical nematode management require the development of new control tactics.

Effector Genes as Pathogen Tools

Whereas *Meloidogyne* spp. are sedentary and induce the formation of feeding sites made up of giant-cells, *Pratylenchus* spp. are migratory parasites that remain mobile upon host invasion and feed by sequential destruction of plant cells. Both nematode genera have evolved intricate molecular interactions with their host plants to overcome defense responses and to establish themselves. At the core of these interactions are nematode effector genes that encode secretions. Effector genes are active in the nematode's esophageal gland cells from where their products are released into host tissue during the infection process. Previous studies reported on the isolation of about 50 effector genes in *M. incognita* (Huang et al. 2003). To date, only very few putative *Pratylenchus* effector genes have been identified (Uehara et al. 2001, Haegeman 2011). Previous studies have demonstrated that some *Meloidogyne* effector genes aid in plant cell wall degradation or modify host cell physiology, but the function of most effector genes is unknown. It is critically important to identify effector genes in *M. chitwoodi* and in *P. penetrans* and to characterize the functions of these genes because this will enable us to devise new control strategies. Effector gene products represent the molecular interface between the nematode and its host, which makes them ideal control targets.

We are pursuing multiple strategies to identify effector genes in *M. chitwoodi* and *P. penetrans*. Previous work has led to the isolation of about 50 effector genes in *M. incognita*,

which is closely related to *M. chitwoodi*. We exploit sequence similarities between these two species to amplify homologous effector genes in *M. chitwoodi* by PCR. In addition, we are mining increasing amounts of genome data for *Meloidogyne* spp. to find effector genes. To clone effector genes in *P. penetrans*, we collect the esophageal gland regions of the nematode and sequence purified cDNA.

Effector Genes as Control Targets

Effector genes enable plant-parasitic nematodes to live a parasitic lifestyle. Their products most likely interact with host plant proteins to modulate host cell physiology. Huang et al. (2006) showed that plants that produce double-stranded (ds) RNA complementary to a *Meloidogyne* effector gene can induce RNA interference (RNAi) in the nematode and are capable of reducing reproduction of *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica* by 63-90%. Our studies show that a similar level of resistance can be achieved against *M. chitwoodi* (P. Dinh and A. Elling, unpublished). RNAi can be used as a new control strategy in its own right by generating plants that produce dsRNA complementary to nematode effector genes (Fig. 1).

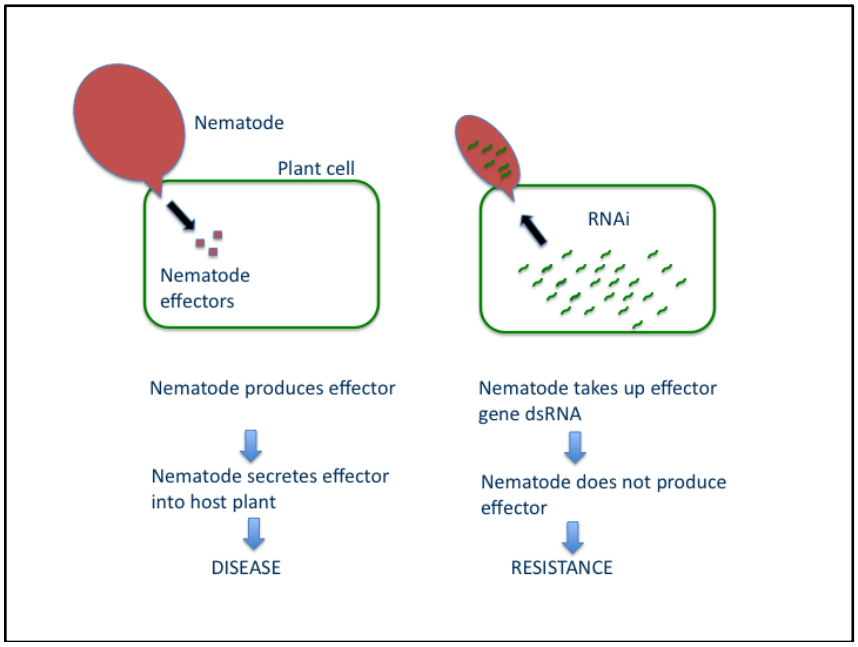

Fig. 1. *Meloidogyne* spp. secrete effector gene products into plant host cells during the infection process. Generating dsRNA complementary to *Meloidogyne* effector genes in plants leads to RNAi-induced deactivation of the respective effector gene in the nematode, thereby conferring resistance.

In addition, RNAi is a powerful tool to identify those effector genes that are indispensable for a successful infection process. If a crucial effector gene is deactivated, a reduced nematode reproduction level can be expected.

Effector gene products most likely interfere with host cell physiology by interacting with host plant proteins. We are using yeast two-hybrid assays to identify potato proteins that interact with *M. chitwoodi* effector gene products and will eventually conduct similar assays for *P. penetrans*. This is important because it will enable us to find and manipulate potato genes that are targets of nematode infection. Improving these genes through breeding could lead to new forms of resistance against *M. chitwoodi* and *P. penetrans* in potato.

Conclusions

M. chitwoodi and *P. penetrans* are serious threats to a sustainable potato production in the Pacific Northwest. Chemical nematode control adds substantially to overall production costs and will become increasingly challenging in the face of new regulations. Identifying and disabling effector genes in *M. chitwoodi* and *P. penetrans* and characterizing the function of these genes during the infection process will enable us to develop new management options for nematode pathogens in potato as part of an integrated pest management program.

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