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The combined approach of strain discovery and the inbred line technique for improving control of *Delia radicum* with *Heterorhabditis bacteriophora*

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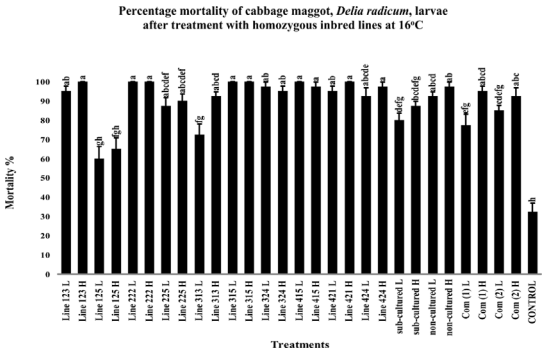
GRAPHICAL ABSTRACT



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ABSTRACT

Entomopathogenic nematodes are potent biocontrol agents but their efficacy can be compromised under unfavorable environmental conditions. For example, cold tolerance can be an essential requirement when utilizing entomopathogenic nematodes in cooler regions. Discovery of new nematode species or strains that are adapted to local conditions is one approach that can be used to enhance efficacy. Once a strain is isolated however, serial culturing can lead to loss of beneficial traits (such as temperature tolerance). Trait deterioration can be eliminated or reduced by creating homozygous inbred lines. In a novel approach to strain improvement, we combined strain discovery (to obtain superior traits) with the inbred line technique (to stabilize traits). The efficacy of ten homozygous inbred lines from a *Heterorhabditis bacteriophora* strain isolated in Ontario, Canada was determined against insect larvae, under low temperature conditions. To assess the impact of serial culturing on nematode performance, two wild-type parent populations (the original 'non-cultured' parent population, and a repeatedly 'sub-cultured' population) were compared with the ten inbred lines. The first experiment evaluated efficacy against *Galleria mellonella* under five constant temperature regimes from 8 to 24 °C. At 16 °C, eight inbred lines and the 'non-cultured' parents demonstrated efficacy against *G. mellonella*. At the warmest temperatures, all nematode treatments were effective. In a subsequent experiment, the efficacy of the nematode lines and two commercial *H. bacteriophora* strains was evaluated at 16 °C against the cabbage maggot, *Delia radicum*. All treatments except one of the inbred lines caused higher levels of infection than the control. The majority of the inbred lines and the 'non-cultured' parental line exhibited superior efficacy compared with the 'sub-cultured'

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population and both commercial strains. This study demonstrates the utility of combining strain discovery with the inbred line approach for improved biological control.

1. Introduction

Performance of entomopathogenic nematodes (EPNs) in Ontario's cool spring and autumn conditions is a key consideration to their successful use for pest control. Poor field efficacy has been reported for an imported strain of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), perhaps as a result of its poor adaptation to cool soil conditions (Kaya, et al., 2006). Low survival levels and inaccurate application times contribute to the lack of control observed under such conditions (Georgis et al., 2006). Efficacy, reproduction and establishment of different EPNs can occur over a wide range of temperatures with most species experiencing reduced infectivity at temperatures below 20 °C (Grewal et al., 1994). There is a need to identify and develop EPN strains that are capable of infecting insect pests at lower temperatures. A straight-forward method to improve biocontrol efficacy, particularly in reference to specific traits, is to isolate new strains that are superior to ones already in use (known as the discovery approach; Shapiro-Ilan et al., 2012). For example, 'cold-adapted' EPN strains have been isolated from soils collected in cool climatic zones (Grewal et al., 2001; Wang et al., 2007; Shapiro-Ilan et al., 2012).

When newly discovered biocontrol organisms are repeatedly cultured in the laboratory or commercial settings they are subject to trait changes, including loss of beneficial traits (e.g., virulence, fecundity, environmental tolerance) (Hopper et al., 1993). Deterioration of beneficial traits has been observed in EPNs (Shapiro et al., 1996; Wang and Grewal, 2002; Bai et al., 2005; Bilgrami et al., 2006). Desirable traits in EPN strains, such as cold tolerance, that are displayed by locally-adapted isolates must be stabilized to avoid a loss in biocontrol efficacy at low temperatures. Generation of homozygous inbred lines is an effective approach for preserving such traits in EPNs (Bai et al., 2005). The creation of inbred lines can enhance genetic stability and permit the production of an elite line in which desirable characteristics are inherently more stable than the parental wild-type (Bai et al., 2005).

Although the discovery approach and the inbred line approach have individually been shown to be viable methods for improvement and stabilization of EPN strains, the two methods have not been implemented concurrently. In this study, we used both methods with the goal of improving and stabilizing a specific biocontrol trait, i.e., improved performance at cool soil temperatures. Our hypothesis was that application of inbred line techniques to a locally adapted strain can increase efficacy characteristics of EPNs at cool temperatures and thus improve opportunities for their successful use under the prevailing climatic conditions of southern Ontario. Using an *H. bacteriophora* strain isolated in Ontario, an array of homozygous inbred lines was created with emphasis on selection of lines that showed enhanced performance at temperatures that would be experienced in soils in southern Ontario during spring and fall.

The study used two insect hosts for proof of concept, larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), and cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae). *Galleria mellonella* is a factitious host for EPN research and production, owing to its high level of susceptibility to various EPN species, and its widespread availability (Shapiro-Ilan et al., 2012), while *D. radicum* is one of the most important insect pests of Brassica crops in Canada (Dixon, 2015). There are three generations of *D. radicum* per year in Ontario (OMAFRA, 2009). The first generation, which is the most damaging, occurs from mid-May to early June, the second from late June to mid-July, and the third from late August to early September (OMAFRA, 2009), which coincide with average temperatures of 15.1, 21.5 and 19.3 °C, respectively (Fig. 1). All life stages of *D. radicum*, with the exception of adults, occur on or in the soil (thus making them a good target for control with EPNs), and the larvae can cause extensive root damage leading to economic losses (Charbonneau and Sears, 2009). In a prior study, Chen et al. (2003) targeted *D. radicum* with EPNs; *S. feltiae* was the only species that proved effective at 10 °C. Three other species (*S. carpocapsae*, *S. arenarium* and *H. megidis*) killed cabbage maggots between 15 and 20 °C, while the lowest effective temperature for *H. bacteriophora* was 20 °C.

2. Materials and methods

2.1. Isolation of EPNs and generation of inbred lines

Sixteen soil samples were collected from residential sites in Goderich and Hanover, Ontario, in June 2011. The nematode extraction process was based on the Whitehead tray method (Whitehead and Hemming, 1965). In brief, 7th instar *G. mellonella* (Vanderhorst Wholesaler Inc., St. Marys, OH) were exposed to soil suspensions prepared from the soil samples in a Petri dish; after 96 h, dead larvae were placed individually on White traps (White, 1927). After 12 days, EPNs were observed in three of the traps and were derived from a single soil sample originally collected from Hanover. These were subsequently identified as *H. bacteriophora* based on their morphological characteristics (Adams and Nguyen, 2002). Their identity was confirmed by a PCR-based protocol (A & L Laboratories, London, Ontario).

Subsequently, inbred lines were generated, according to the methods of Glazer et al. (1991) and Bai et al. (2005). Surface sterilized infective juveniles (IJs) were exposed to a monoxenic culture of the

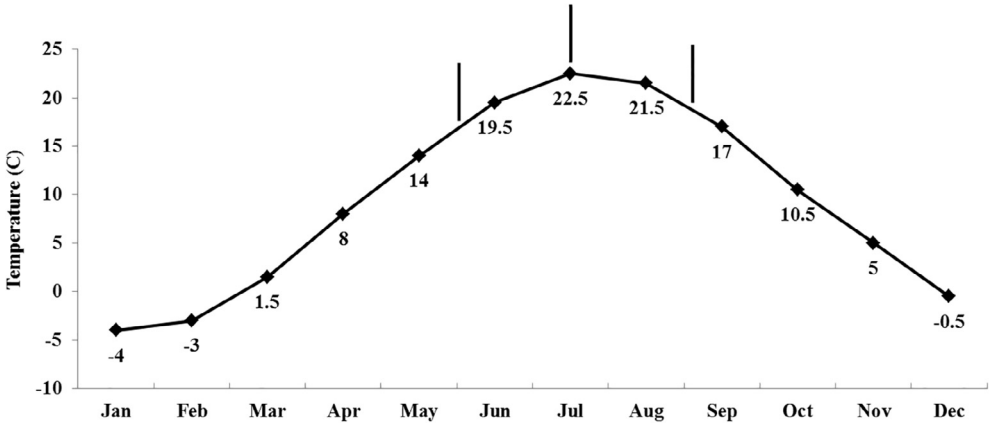

Fig. 1. The average monthly temperature (°C) in Toronto, Ontario, based on climate data gathered from 1995 to 2014 (http://climate.weather.gc.ca, 2015). The three generations of cabbage maggot, *Delia radicum*, that occur in southern Ontario are represented by the bars above the graph.