Cost-effective culturing of the yellow mealworm and greater wax moth larvae, for the in vivo production of a biological control agent: entomopathogenic nematodes

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The establishment and maintenance of insect colonies is vital to the advancement of biological insect-control techniques and has lately especially been used for sterile insect release (SIR) purposes. The first step in the in vivo production process of entomopathogenic nematodes (EPNs) for use as biological control agents is the rearing of susceptible hosts. In order to produce EPNs of consistent quality, a continuous source of host insects reared on a standardised diet is required. Wax moth larvae (Galleria mellonella L.) and mealworms (Tenebrio molitor L.) were selected as hosts in the current study for the two endemic EPN isolates used, Heterorhabditis zealandica and H. bacteriophora. Each host was reared on a specific diet under optimal ambient conditions. The culture method that was used is easily reproducible, practical and can produce insect hosts on a small scale over an indefinite period of time.

Introduction

Annually, insect pests cause serious damage to agricultural crops, which leads to substantial financial losses in agriculture worldwide. In order to control such pests, humankind has tended to rely heavily on synthetic chemical pesticides. Although chemical pesticides have played an important role in controlling agriculturally important pests, their use has also led to pesticide resistance, secondary pest outbreaks, pesticide residues on crops, and health risks to animals and humans. Pesticide availability is also becoming increasingly restricted, as a result of more stringent safety requirements, which have led to the banning of many products. The negative consequences, and the current status, of synthetic compounds have contributed to an increased interest in adopting natural approaches. Such approaches are based on the development and incorporation of more environmentally benign alternatives in pest management practices. Biological control is one such alternative and, as a component of a pest management programme EPNs can be periodically introduced to maintain host population levels below what they would be in the absence of the nematodes.

Entomopathogenic nematodes

EPNs are naturally occurring, non-segmented, colourless, elongated, insect-parasitic roundworms, living in a variety of soil types and possessing the ability to infect over 200 insect hosts under laboratory conditions. The free-living, non-feeding infective juvenile (IJ), which is the invasive stage in the life cycle of EPNs, is used for insect control purposes. Once the IJ comes into contact with the host, depending on the specific species, it enters the insect through natural openings (mouth, anus, spiracles) or the body wall. The nematodes feed on the bacteria cells and metabolised host tissue, developing and reproducing within the insect cadaver for as long as nutrients are abundant. When all the nutrients have been consumed, the EPNs exit the cadaver in search of a new susceptible host.

Apart from EPNs being especially efficacious against soil-borne pests, they also show great potential for suppressing pests above ground via foliar application. In addition to EPNs being able to suppress pests within one to two days, they also have the ability to persist for two to three weeks in the field.

Isolates of Heterorhabditis bacteriophora and Heterorhabditis zealandica, which have been collected during surveys conducted in South Africa, have been identified as being very effective against key insect pests of deciduous and citrus fruits. The insect hosts that were cultured in the current study were selected due to their proven high susceptibility to the two above-mentioned nematode species.

EPN production

One of the prerequisites for a biological control agent to be successful is the need to be capable of replicating it artificially in high numbers. EPNs can be cultured using either in vivo or in vitro technology.

In vivo technology involves the inoculation of a susceptible insect host with the desired nematode to be replicated. As the insect host serves as a bioreactor, in which nematodes multiply, a large number of hosts are required for in vivo mass production of EPNs. High-quality nematodes are produced in such a manner. The number of nematodes that is produced in vivo is sufficient for use by the local market, in order to supply an inoculum for small-scale field trials and for the maintenance of laboratory cultures. In vivo production is a low-tech, albeit labour-intensive, process and is easily implemented in research laboratories, cooperations, cottage industries, and in such developing countries as South Africa, where labour is still relatively inexpensive compared to labour costs in first-world countries.

Compared to in vivo production, in vitro production is a highly mechanised, capital-intensive, high-tech process, which is ideal for the commercial mass production of EPNs. As soon as higher numbers of nematodes are required, for example with orchard application, in vitro production is a more practical option, as large numbers of IJs can be efficiently produced in fermenting tanks. Nematodes have been commercially developed in North Amer-
ica, Europe, Australia and Asia for the control of a vast array of pests, ranging from pests occurring in greenhouses to those occurring on golf course turfs. Some of the pests concerned include citrus root weevils in citrus, black vine weevils in nurseries, mole crickets on turf grass, peach borer and codling moth on apples, and black cutworms.

Biotechnological equipment and technical expertise regarding in vitro EPN production is, unfortunately, limited in South Africa and the commercial in vitro production of nematodes has yet to see the light of day in the country. In the interim, in vivo culturing methods are used in laboratories for experiments and small-scale field trials. In South Africa, research into the use of EPNs has been undertaken regarding the control of codling moth; mealy-bug; the banded fruit weevil; and false codling moth. Specific nematode isolates have been identified as promising biological control agents against the pests.

Hopefully, in vivo production can be used as a stepping stone that could soon lead to the development of in vitro culturing of indigenous nematode species for the commercial market in South Africa.

**Insect hosts** The most general and widely used host for in vivo EPN production is the greater wax moth (Galleria mellonella L.) larvae. Wax moth larvae are a major pest in apiaries, causing severe damage to stored and unattended honeycombs. In addition to being highly susceptible to nematodes, wax moth larvae are widely available, and can easily be reared on artificial diets within a relatively short space of time. The late-instar larvae produce sufficient numbers of nematodes to make their use feasible for in vivo production. Another promising host for nematode production is the yellow mealworm, Tenebrio molitor (L.). Mealworms are general decomposers and pests on poultry farms and in grain storage facilities. In most aspects, they measure up to wax moth larvae as being stellar hosts, yet they are less susceptible to nematodes when compared to such larvae, and also tend to produce fewer nematodes per host.

**Rearing of wax moth larvae** Wax moth eggs and larvae obtained from the initial laboratory colony were placed on top of an artificial diet in well-ventilated plastic containers measuring 11 x 11 x 7.5 cm (length x width x height). The artificial diet consisted of 118 g wheat flour, 206 g wheat bran, 118 g milk powder, 88 g brewer’s yeast, 24 g wax powder, 175 ml honey and 175 ml glycerol. The plastic containers were modified by inserting mesh screen into the lid to facilitate air and heat exchange, with the aim of avoiding condensation. The life cycle of wax moth larvae includes an egg, a larva, pupa and moth stage, and development took place at an average temperature of 26°C and at an average relative humidity (RH) of 55%. Moths were regularly removed from the diet as they hatched one to two weeks after pupation. They were then placed together in glass jars containing pleated wax paper, which served as an oviposition site for the female moths. The moths, which started laying eggs three to five days after emergence, had an average life span of one to two weeks at 26°C. Eggs were regularly removed from the paper with a razor blade and transferred to a plastic container, continued on page 71.
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Mealworm adults being placed on top of the diet in wooden boxes.

containing 1 kg of fresh diet. Eggs hatched within four to five days after being laid. The diet was replenished as needed to ensure the availability of sufficient nutrients for the larvae. The larval stage lasted between 20-40 days, after which time the larvae not used in experiments pupated, and the life cycle, which took six to seven weeks, was repeated.

Rearing of mealworms

Mealworm larvae, which were obtained from a laboratory culture, were reared on wheat bran as culture medium in various sized wooden culture boxes, which were covered with a solid lid to limit the amount of light penetration. Bran was frozen before use to prevent mite infection. Sufficient wheat bran (1 kg) was added to facilitate burrowing for larvae and adult beetles. Carrots, wiped with 96% ethanol prior to use, were placed on top of the diet to serve as water source, and were replenished as required during the life cycle, which varied between 12-16 weeks. Wooden culture boxes containing the egg, larva, pupa and beetle stages of MW were placed in a temperature-regulated room at an average temperature of 26°C and an RH of 55%. Depending on the size of the colony, bran was replenished once every two weeks and carrots were added every week. At least once every three months, or as soon as the diet seemed to become moist, beetles and larvae were separated from eggs and frass, using a sieve of 1200 µm. Fresh diet and carrots were then added to the culture box, containing beetles and larvae. Frass containing the eggs was placed in a separate container, to which fresh diet and carrots were also added, making it available for consumption by the next generation of mealworms.

During periods that mealworm and wax moth larvae were not needed in large numbers for experiments, the culture boxes were moved to a 14°C culture room to slow down the developmental rate of the hosts concerned.

Conclusion

The aim of the culture method used in the current study was to ensure a continuous supply of sufficient numbers of high-quality wax moth larvae and mealworms. The aim was successfully achieved, with the insects concerned being available, in various life stages, throughout the study. Basic equipment was used and the diets selected were also cost-efficient. Although slight contamination problems were experienced in initial mealworm diets, due to the addition of carrots as water source, the problem could be overcome by removing the carrots before rotting or by replacing them with an alternative water source, such as wetted paper towels. Sufficient numbers of mealworms were still produced, even though moderate contamination occurred. However, the prevention of contamination through the implementation of effective sanitary measures is key to a successful rearing programme.

The in vivo production of EPNs for the control of insect pests has been extensively studied. However, enhancing and streamlining each step in the production process could contribute towards creating a more effective, cost-efficient and practical culturing method, specifically customised for EPNs that are endemic to South Africa.

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co-operation with the Forelle Producers Association (FPA). The correct harvest maturity standards would need to be identified and timeously distributed to all those participating in the programme, and suitable early orchards that meet the delayed harvesting specifications, must be identified and monitored. Although levels of astringency were low in the orchards selected for the 2011 trial, as this disorder is predominantly found in less mature fruit, a few consumers did complain about the fruit being astringent. Future orchards must therefore be selected that do not have a history of astringency. Delaying harvest for 2 to 3 weeks is not without its risks as fruit may experience a slight loss in red blush colour, and may be more prone to abscission in strong winds. For this reason, the orchards intended for short term storage would need to be carefully selected and monitored. A system must be set in place to ensure that only Forelle fruit that have met the above criteria and have been subjected to SmartFresh™ are exported.

Suitable target markets and retail partners would need to be identified and there must be a strategy to sell or keep the surplus fruit that do not make the export grade. Dispensation during semi-commercial trialing would need to be applied for, and most importantly, there must be continued feedback from retailers and supermarkets regarding consumer acceptance and/or complaints.

Reference


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