Chapter 3

Entomopathogenic Nematodes in Pest Management

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Additional information is available at the end of the chapter

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Abstract

The definition "biological control" has been used in different fields of biology, most notably entomology and plant pathology. It has been used to describe the use of live predatory insects, entomopathogenic nematodes (EPNs) or microbial pathogens to repress populations of various pest insects in entomology. EPNs are among one of the best biocontrol agents to control numerous economically important insect pests, successfully. Many surveys have been conducted all over the world to get EPNs that may have potential in management of economically important insect pests. The term "entomopathogenic" comes from the Greek word *entomon* means insect and *pathogenic* means causing disease and first occurred in the nematology terminology in reference to the bacterial symbionts of *Steinernema* and *Heterorhabditis*. EPNs differ from other parasitic or necromenic nematodes as their hosts are killed within a relatively short period of time due to their mutualistic association with bacteria. They have many advantages over chemical pesticides are in operator and end-user safety, absence of withholding periods, minimising the treated area by monitoring insect populations, minimal damage to natural enemies and lack of environmental pollution. Improvements in mass-production and formulation technology of EPNs, the discovery of numerous efficient isolates and the desirability of increasing pesticide usage have resulted in a surge of scientific and commercial interest in these biological control agents.

Keywords: biological control, safety, entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*

1. Entomopathogenic nematodes

1.1. General information of entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are soil-inhabiting, lethal insect parasites that belong to the Phylum Nematoda from the families Steinernematidae and Heterorhabditidae, and they have proven to be the most effective as biological control organisms of soil and above-ground pests [1, 2]. They have been known since the seventeenth century [3], but it was only in the 1930s that serious care was given by using nematodes for pest control.

So far, the family Steinernematidae is comprised of two genera, *Steinernema* Travassos, 1927 [4] (Poinar, 1990) and *Neosteinernema* (Nguyen and Smart, 1994) [5]. *Neosteinernema* contains only one species *Neosteinernema longicurvicauda* that isolated from the termite *Reticulitermes flavipes* (Koller). The family Heterorhabditidae contains only one genus, *Heterorhabditis* Poinar, 1976 [6].

EPNs are mutually associated with bacteria of the family Enterobacteriaceae; the bacterium carried by Steinernematidae is usually a species of the genus *Xenorhabdus*, and that carried by Heterorhabditidae is a species of *Photorhabdus*. The third juvenile stage of EPNs is referred to as the "infective juvenile" (IJ) or the "dauer" stage. IJs of both genera release their bacterial symbionts in the insect host body and develop into fourth-stage juveniles and adults. The insects die mainly due to a septicemia. Sometimes a bacterial toxaemia precedes the resulting septicemia [7].

Infective juvenile is the only free-living stage and can survive in soil for several months until susceptible insects are encountered. IJs locate and infect suitable insect hosts by entering the insect host through the mouth, anus, spiracles or thin parts of the host cuticle. After infection, the symbiotic bacteria are released into the insect haemocoel, causing septicaemia and death of the insect [1, 8]. When an insect host is infected in the soil by an EPN, development and reproduction within the cadaver can take 1–3 weeks [9].

Surveys for EPNs have been conducted in temperate, subtropical and tropical regions and found that EPNs have a worldwide distribution; the only continent where they have not been found is Antarctica [10]. Soil texture, temperature and host availability are thought to be important factors in determining their distribution [11–13].

Nearly 70 valid species of *Steinernema* [14–16] and 25 species of *Heterorhabditis* [17, 18] have been described worldwide and still surveys for EPNs have been conducted in many parts of the world.

1.2. Biology and life cycle of entomopathogenic nematodes

Through all nematodes studied to control insects, the families Steinernematidae and Heterorhabditidae have made a sensation and information about them is increasing exponentially. Steinernematids and Heterorhabditids from these families have similar life cycles, and the only difference between the life cycles of *Heterorhabditis* and *Steinernema* is occurred in the first generation. *Steinernema* species are amphimictic; this means that for successful reproduction

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they require the presence of males and females, whereas *Heterorhabditis* species are hermaphroditic and able to reproduce in the absence of conspecifics.

Both nematode genera reproduction is amphimictic in the second generation [4]. However, a hermaphroditic Steinernematid species was isolated from Indonesia [19]. Only the free-living, IJ stage is able to target insect host and the only form found outside of the host. EPNs occur naturally in soil and locate their host in response to carbon dioxide, vibration and other chemical cues, and they react to chemical stimuli or sense the physical structure of insect's integument [1].

IJs penetrate the host insect via the spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle, and then enter into the haemocoel [20]. IJs release cells of their symbiotic bacteria from their intestines into the haemocoel. The bacteria multiply rapidly in the insect hemolymph, provide nematode with nutrition and prevent secondary invaders from contaminating the host cadaver, and the infected host usually dies within 24–48 hours by bacterial toxins.

Nematodes reproduce until the food supply becomes limiting at which time they turn into IJs. The progeny nematodes go through four juvenile stages to the adult. Based on the available resources, one or more generations may occur within the host cadaver, and a great number of IJs are released into environment to infect other host insects and continue their life [1].

The insect cadaver becomes red if the insects are killed by Heterorhabditids and brown or tan if killed by Steinernematids (**Figure 1**). The colour of the insect host body is indicative of the pigments produced by the monoculture of mutualistic bacteria growing in the host insects [1].

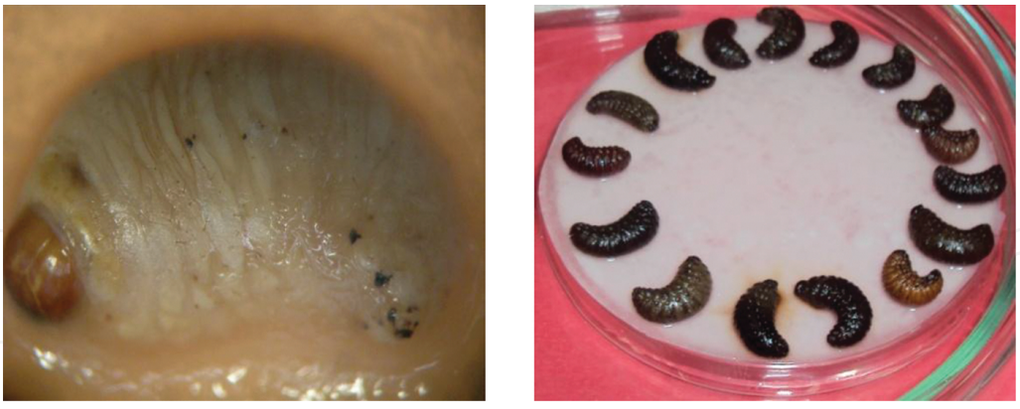

Figure 1. Different colours of the dead *Curculio nucum* larvae on white traps after EPNs infection.

The foraging strategies of EPNs change between species, and they use two main foraging strategies: ambushers or cruisers [21]. *Steinernema carpocapsae* is an example of ambushers, which have an energy-conserving approach and lie in wait to attack mobile insects (nictitating) in the upper layer of the soil. *Steinernema glaseri* and *Heterorhabditis bacteriophora* are examples of cruisers are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. But they are also successful

to attack white grubs (Scarab beetles), which are less mobile. Other species, such as *Steinernema feltiae* and *Steinernema riobrave*, use an intermediate foraging strategy (combination of ambush and cruiser type) to find their host.

Selection of an EPN to control a particular pest insect is based on various factors: the nematode's host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy. The most critical factors are moisture, temperature, pathogenicity for the targeted pest insect and foraging strategy [1, 22–24]. The activity, infectivity and survival of EPNs can be profoundly influenced by soil composition, through its effects on moisture retention, oxygen supply and texture [25–27].

Within a favourable range of temperatures, adequate moisture and a susceptible host, those EPNs with a mobile foraging strategy (cruisers and intermediate foraging strategies) could be considered for use in subterranean and certain above-ground habitats (foliar, epigeal and cryptic habitats). Those EPNs with a sit and wait foraging strategy (ambushers) will be most effective in cryptic and soil surface habitats [28].

1.3. Advantages of entomopathogenic nematodes

These nematodes have many advantages; EPNs and their associated bacterial symbionts have been proven safe to warm-blooded vertebrates, including humans [29, 30]. Cold-blooded species have been found to be susceptible to EPNs under experimental conditions at very high dosages [31, 32]. However, under field conditions, the negative results could not be reproduced [33, 34].

Most biological agents require days or weeks to kill the host, yet nematodes can kill insects usually in 24–48 hours. They are easy and relatively inexpensive to culture, live from several weeks up to months in the infective stage, are able to infect numerous insect species, occur in soil and have been recovered from all continents except Antarctica [1, 35].

Foliar applications of nematodes have been successfully used to control the quarantine leaf-eating caterpillars as *Tuta absoluta*, *Spodoptera littoralis*, *Helicoverpa armigera*, *Pieris brassicae* on several crops and have the potential for controlling various other insect pests. Application of EPNs does not require masks or other safety equipment like chemicals. EPNs and their associated bacteria have no detrimental effect to mammals or plants [29, 30, 36].

2. Use of entomopathogenic nematodes

Potential of EPNs as insecticidal agents has been tested against a wide range insect species by many researchers all over the world. They have been used with different success against insect pests occurred in different habitats. Much success has been obtained against soil-dwelling pests or pests in cryptic habitats such as inside galleries in plants where IJs find excellent atmosphere to survive and protect themselves from environmental factors. Commercial use of EPNs against some pest insects is given in **Table 1**.

Crops (targeted)	Pest common name	Pest scientific name	Effective nematodes[b]
Artichokes	Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Vegetables	Armyworm	Lep: Noctuidae	Sc, Sf, Sr
Ornamentals	Banana moth	*Opogona sacchari*	Hb, Sc
Bananas	Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Turf	Billbug	*Sphenophorus* spp. (Col: Curculionidae)	Hb, Sc
Turf, vegetables	Black cutworm	*Agrotis ipsilon*	Sc
Berries, ornamentals	Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hd, Hm, Hmeg, Sc, Sg
Fruit trees, ornamentals	Borer	*Synanthedon* spp. and other sesiids	Hb, Sc, Sf
Home yard, turf	Cat flea	*Ctenocephalides felis*	Sc
Citrus, ornamentals	Citrus root weevil	*Pachnaeus* spp. (Col: Curculionidae)	Sr, Hb
Pome fruit	Codling moth	*Cydia pomonella*	Sc, Sf
Vegetables	Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Vegetables	Corn rootworm	*Diabrotica* spp.	Hb, Sc
Cranberries	Cranberry girdler	*Chrysoteuchia topiaria*	Sc
Turf	Crane fly	Dip: Tipulidae	Sc
Citrus, ornamentals	Diaprepes root weevil	*Diaprepes abbreviatus*	Hb, Sr
Mushrooms	Fungus gnat	Dip: Sciaridae	Sf, Hb
Grapes	Grape root borer	*Vitacea polistiformis*	Hz, Hb
Iris	Iris borer	*Macronoctua onusta*	Hb, Sc
Forest plantings	Large pine weevil	*Hylobius abietis*	Hd, Sc
Vegetables, ornamentals	Leafminer	*Liriomyza* spp. (Dip: Agromyzidae)	Sc, Sf
Turf	Mole cricket	*Scapteriscus* spp.	Sc, Sr, Sscap
Nut and fruit trees	Navel orangeworm	*Amyelois transitella*	Sc
Fruit trees	Plum curculio	*Conotrachelus nenuphar*	Sr
Turf, ornamentals	Scarab grub[c]	Col: Scarabaeidae	Hb, Sc, Sg, Ss, Hz
Ornamentals	Shore fly	*Scatella* spp.	Sc, Sf
Berries strawberry	Root weevil	*Otiorhynchus ovatus*	Hm
Bee hives	Small hive beetle	*Aethina tumida*	Hi, Sr
Sweet potato	Sweetpotato weevil	*Cylas formicarius*	Hb, Sc, Sf

[a]Nematodes listed provided at least 75% suppression of these pests in field or greenhouse experiments.
[b]Abbreviations of nematode species; Hb: *Heterorhabditis bacteriophora*, Hd: *H. downesi*, Hi: *H. indica*, Hm: *H. marelata*, Hmeg: *H. megidis*, Hz: *H. zealandica*, Sc: *Steinernema carpocapsae*, Sf: *S. feltiae*, Sg: S. *glaseri*, Sk: *S. kushidai*, Sr: *S. riobrave*, Sscap: *S. scapterisci*, Ss: *S. scarabaei*.
[c]Efficacy against various pest species within this group varies among nematode species.

Table 1. Use of entomopathogenic nematodes as biological control agents[a] [37].

2.1. Efficacy of entomopathogenic nematodes against tomato leaf miner *Tuta absoluta*

In our laboratory, we investigated the use of native EPN isolates to control various pest insects, and one of these pests was tomato leaf miner. The tomato leafminer, *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a very devastating pest and was first recorded in 2009 in the Urla District of Izmir Province in Turkey [38]. It has been a serious problem to tomato production in Çanakkale since the first detection in our country [39]. *T. absoluta* can attack all parts and stages of the tomato plant, overwinter in the egg, pupal or adult stage and can cause up to 100% losses in tomato crops [40].

Since its dispersal in the 1970s, chemical control has been the main method to control *T. absoluta*. Producers have tried to decrease its damages by using insecticides twice a week during a cultivation period, sometimes every 4–5 days/season with 8–25 sprays [41]. Although with the many applications of chemicals, effective control is difficult due to the behaviour of these mine-feeding larvae.

Moreover, the use of pesticides in plant production has numerous disadvantages as pesticide residues on human health and on the environment so biological control may be considered as an alternative method to chemical control [42]. In this respect, EPNs can be an alternative to chemicals. The aims of the work were to determine the efficacy of native EPN isolates against *T. absoluta* in tomato field and to reduce the use of pesticides.

2.2. Materials and methods

2.2.1. Entomopathogenic nematodes culture

Four native species of nematodes: *Steinernema affine* (Bovien) (isolate 46) *S. carpocapsae* (Weiser) (isolate 1133), *S. feltiae* (Filipjev) (isolate 879) and *H. bacteriophora* (Poinar) (isolate 1144), were tested against *T. absoluta* larvae. Each isolates was reared in the last instar of wax moth larvae *Galleria mellonella* L., which is the most commonly used insect host for in vivo production of EPNs because of its rich nutrient source available in body and easy to multiply in economical diet source [43, 44].

Nematode-infected *G. mellonella* larvae were placed on white traps [45] at 25°C and IJs that emerged from cadavers were harvested.

2.2.2. Tuta absoluta culture

Larvae, pupae and adults of *T. absoluta* used in the trials were obtained from infested tomato fields in Çanakkale. They reared in wooden rearing cages (50 × 50 × 50 cm) on tomato plants at 25 ± 1°C, 65 ± 5% RH, with a 16:8 L:D photoperiod in climate room.

2.2.3. Field trials

Field trials were carried out in the training and research area of Agriculture Faculty in Çanakkale between 2012 and 2013. In both seasons, nearly 1000 m^2 area was cultivated with tomato and seedlings were controlled periodically and closed by a cage when they reached 20

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cm height. Each tomato plant was grown in a single cage (50 × 50 × 50 cm). After 30 days, two males and two females were put into each cage.

EPNs were applied at dusk to utilise the higher air humidity for the nematodes with a conventional airblast sprayer at a rate of 50 IJs/cm^2. Tomato plants remained wet in cages after application for 2 hours and that provides EPNs enough time with perfect condition to find and infect the target pest. The experiment was carried out with two replicates per nematode species and exposure day and repeated twice.

After releasing the adults of *T. absoluta*, EPNs were sprayed on tomato plants at the 7th, 14th and 21st days. Tomato plants were cut from the soil line at the 3rd, 5th 7th, 9th, 11th, 13th and 15th days after EPN applications and analysed to determine the mortality of *T. absoluta*. Dead *T. absoluta* larvae were immediately dissected and checked for nematode infection (**Figure 2**). EPNs most likely entered feeding canals in the leaves of tomatoes. Many larvae of *T. absoluta* died inside these galleries, which indicate that IJs were able to find and infect them.

Figure 2. Emerged EPNs from infected *Tuta absoluta* larvae.

2.3. Results

The efficacy of EPNs in field in 2012 changed between 0 and 90.7 ± 1.5%. The least efficient species was *S. affine* and the most efficient species was *S. feltiae* with the mortality of 39.3 ± 1.5% and 90.7 ± 1.5%, respectively. *S. affine* caused 0–39.3 ± 1.5% mortality and found as the least efficient species. *S. carpocapsae* caused 0–43.7 ± 1.5% mortality, while *S. feltiae* caused 0–90.7 ± 1.5% mortality. *H. bacteriophora* caused 0–81 ± 3.5% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in tomato field in 2012.

The efficacy of EPNs in field in 2013 changed between 0 and 94.3 ± 2.0%. The least efficient species was *S. affine* and the most efficient species was *S. feltiae* with the mortality of 43.7 ± 2.3% and 94.3 ± 2.0%, respectively. *S. affine* caused 0–43.7 ± 2.3% mortality and was the least efficient species. *S. carpocapsae* caused 0–49.3 ± 2.4% mortality, while *S. feltiae* caused 0–94.3 ± 2.0% mortality. *H. bacteriophora* caused 0–83.0 ± 2.1% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in field in 2013.

2.4. Discussion

The tomato leafminer, *T. absoluta*, is one of the most important lepidopteran moth associated with tomato plants and because of its biology and behaviour, it is difficult to control. Effective chemical control of *T. absoluta* is not possible because it feeds internally within the plant tissues. Resistance to insecticides is another significant problem in chemical control of this pest because of its high reproduction capacity, short generation cycle and intensive use of insecticides [46–50].

Pesticides are so widely used and that destroys populations of natural enemies and consequently decreases biological control of *T. absoluta*. Because of these negative effects of insecticides, other approaches need to be considered seriously for this devastating pest.

Some insects can be controlled by a combination of methods, which are not totally effective when used alone. *T. absoluta* is one of these insects, which requires more than one method to be controlled successfully. For this reason, integrated pest management (IPM) programmes are continuously being progressed in different countries to control infestations of tomato leaf miner. EPNs have been considered as potential biocontrol agents for leafminers in recent years [50]. They can be applied, in combination with other biological and chemical pesticides, fertilisers and soil amendments and in the form of adjuvants or antidesiccants [51, 52].

Various studies about EPNs have been conducted all over the world, but only few research has been carried out on the efficacy of EPNs against *T. absoluta*. This is the first study conducted both in çanakkale and in Turkey based on the efficacy of native EPN isolates to *T. absoluta* in a tomato field.

The efficacy of the three EPNs after foliar application to potted tomato was tested under greenhouse conditions. High larval mortality (78.6–100%) and low pupal mortality (<10%) in laboratory were reported. In the leaf bioassay, high larval parasitisation (77.1–91.7%) was recorded. In the pot experiments, it was found that nematode application decreased insect infestation of tomato by 87–95%. These results showed the suitability of EPNs to control *T. absoluta* [53].

The efficacy of soil treatments of three native EPNs (*S. carpocapsae, S. feltiae* and *H. bacteriophora*) against *T. absoluta* larvae, pupae and adults was determined under laboratory conditions in another study [54]. The effect of three commonly used insecticides against *T. absoluta* was also evaluated in the survival, infectivity and reproduction of these EPNs. When the larvae dropped into the soil to become pupa, soil application of nematodes resulted in a high larval mortality: 100, 52.3 and 96.7% efficacy for *S. carpocapsae, S. feltiae* and *H. bacteriophora*, respectively. No mortality of pupae was recorded, and mortality of adults emerging from soil was 79.1% for *S. carpocapsae* and 0.5% for *S. feltiae*. An insignificant effect of the insecticides tested was reported on nematode survival, infectivity and reproduction. No sublethal effects were observed. These findings proved that larvae of *T. absoluta*, falling from leaves following insecticide application, could be favourable hosts for nematodes, thereby increasing their concentration and persistence in the soil.

The efficacy of *S. feltiae, S. carpocapsae* and *H. bacteriophora* was evaluated against larvae of *T. absoluta* inside leaf mines in tomato leaf discs by means of an automated spray boom. They

reported that all EPNs used in the study were effective to all four larval instars of *T. absoluta* but caused higher mortality in the later instars (fourth instar: 77.1–97.4%) than in the first instars (36.8–60.0%). *S. feltiae* and *S. carpocapsae* showed better results than *H. bacteriophora*. *S. carpocapsae* and *H. bacteriophora* performed better at 25°C (55.3 and 97.4% mortality, respectively) than at 18°C (12.5 and 34.2% mortality, respectively), while *S. feltiae* caused 100% mortality at both temperatures. Their results demonstrated that under laboratory conditions, *S. feltiae* and *S. carpocapsae* showed effective performance against the larvae of *T. absoluta* inside tomato leaf mines [55].

Our results agree with other reports showing that larvae of *T. absoluta* were highly susceptible to the EPNs tested and these EPNs can be used as efficient biological control agents against *T. absoluta*. All EPNs used in the study showed efficacy at different rates against *T. absoluta*. They were able to find and infect *T. absoluta* larvae both inside and outside of the tomato leaf. According to these findings, it could be suggested that EPNs have a great potential to use as biocontrol agents for the management of *T. absoluta*.

It should be noted that to understand their life cycles and functions, match the correct species of EPNs with the correct species of insect pests, apply them under optimum environmental conditions, such as soil temperature, soil moisture, angle of sun rays, and apply only with compatible pesticides are the keys to success with EPNs.

3. Conclusions

Biological control is an action that involves the use of natural enemies of insect pests to increase negative effects of insect pest as destroying important crops and plantation, plant growth destruction or development infections caused by pests [56].

Advantages	Disadvantages
Broad host range of pest insect	High cost in production
Able to seek or ambush the host and can kill rapidly the host	Lack of labour, knowledge and skills required in nematology
Mass produced by *in vivo* and *in vitro* (solid and liquid culture medium)	Limited shelf life and refrigerated storage required
Can be used with conventional application equipment	Difficulties in formulation and quality control
Safety for all vertebrates, most non-target invertebrates and the food sources	Environmental limitations; for survival and infectivity adequate moisture and temperatures are needed, sensitivity to UV radiation, lethal effect of several pesticides (nematicides, fumigants and others) lethal or restrictive soil properties (high salinity, high or low pH, etc.)
Little or no registration required	

Table 2. Advantages and disadvantages of entomopathogenic nematodes [58].

EPNs are a group of soil-dwelling organisms that attack soilborne insect pests that live in, on or near the soil surface and can be used effectively to control economically important insect pests. Different nematode species and strains exhibit differences in survival, search behaviour and infectivity, which make them more or less suitable for particular insect pest control programmes [57]. As the other biological control agents, also EPNs have advantages and disadvantages (**Table 2**).

There is a great interest in finding wild populations to obtain new species and strains for possible use in biological control. The use of EPNs is one potential non-chemical approach to control insect pests. EPNs are widely spread geographically and have many hosts. They are currently used as biological control agents in many studies to control several important insect pests worldwide [59–61].

It is highlighted that there is a need for more in-depth basic information on EPNs biology, including ecology, behaviour and genetics, to help understand the underlying reasons for their successes and failures as biological control organisms. Most appropriate nematode species/strain, abiotic factors such as soil type, soil temperature and moisture are important for getting success [1].

Proper match of the nematode to the host entails virulence, host finding and ecological factors are essential before application to the field. Matching the appropriate nematode host-seeking strategy with the pest is essential, because poor host suitability has been the most common mistake occurred in application of EPNs [62]. Also application strategies, such as field dosage, volume, irrigation and appropriate application methods, are very important. Furthermore, plant morphology and phenology must be considered in predicting whether nematodes are viable control candidates [63].

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