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RESEARCH ARTICLE

COMPATIBILITY OF ENTOMOPATHOGENIC NEMATODES WITH INSECTICIDES IN IPM SYSTEM

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ABSTRACT

Entomopathogenic nematodes (EPNs) are potential biocontrol agent against many economically important crop pests. Combining the use of biological control agent with chemical insecticide in IPM programme leads to reduced the environmental risk as well as management costs. The author review different aspects of compatibility or interaction of insecticide with EPNs which will show promise to contain pest infestation in agriculture.

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INTRODUCTION

The widespread use of pesticides against potential pests has resulted in the appearance of pesticide resistance in many pests, phytotoxicity, pesticide residue problems on plant products, growing cost of the plant production and overall negative environmental impacts on human and animal health (Dalvi *et al*., 2011). It has been suggested that combining reduced rate of insecticides with bio control agent could achieve adequate control insect pests while reducing the adverse effects of insecticides in Integrated Pest Management (IPM) system (Alfred and Grewal, 2004). Entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* and *Steinernema* under family Heterorhabditidae and Steinernematidae, respectively are potential biological control agents against many insect pests .EPNs may contribute more if integrated with other methods of control than their use solely as biocontrol agents. It has thus become very important to know more about which insecticides help the nematodes IPM system. Moreover, chemical insecticide manufacturing industries do not test product toxicity to entomopathogens, just like for predators and parasitoids. It may reduce the dependence on chemical insecticides and thus contribute to slowing down the development of insecticide resistance and preventing adverse effects on environment.

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Enabling the tank mixing of EPNs with other control products in a specific IPM programme could lead to increased control of the target pest, with greater cost-effectiveness, and with a reduction in application time required (Koppenhofer and Grewal, 2005).

Compatibility Study: Entomopathogenic nematodes are often applied to sites and ecosystems that routinely receive other inputs like chemical pesticides, wetting agents, fertilizers, and soil amendments. Most of the studies of the compatibility (survivability/activity/viability) of entomopathogenic nematodes (EPNs) with insecticides have been conducted as laboratory bioassays with direct exposure of nematodes in aqueous solutions. However studies on penetration rate, infectivity, reproduction rate of EPNs are conducted in insect hosts following pesticide exposure (Table1). Nictating behavior appears to be a better indicator than movement for screening pesticides for compatibility with nematodes. Nematodes could be successfully mixed with chemicals that enhance the nictating behavior of infective juveniles. It is still necessary to test the commonly used pesticides in the area where EPNs are anticipated to be used. Nematode response to insecticide residues resulting from foliar application to plants infested with foliar pest should also be investigated.

Interaction Study: The chemical substances employed to control a single pest can reduce the efficacy of EPNs, or may be synergistic, since at sub-lethal doses they can cause stress on the insect pest and provide better control by the nematode (Kaya *et al*., 1995; Koppenhofer *et al*., 2000).

It has been observed that exposure to certain chemicals may stimulate nematode movement and enhance host finding behavior and penetration of the host (Ishibashi and Takii, 1993). In field trials, a mixed application of *Steinernema carpocapsae* with certain insecticides, viz., diazinon, fenitrothion, dichlorvos, oxamyl, acephate, permethrin has provided more effective insect control than separate applications of each. Such chemicals may stimulate passive or inactive nematodes and thereby enhance their infectivity against the target insects. Helminth parasites possess a number of mechanisms for detoxification of harmful xenobiotics. Helminths also use activity of the cytochrome P450 system (Kotze, 1997). Piperonyl-butoxide acts as a synergist by inhibiting the cytochrome P450-mediated metabolism of the insecticide (Jones 1998). *S. feltiae* may possess only restricted possibilities for metabolizing this chemical compound, piperonyl-butoxide using the cytochrome P450 system. Combinations of insecticides and insect parasitic nematodes have a synergistic effect on nematode infection rates against white grubs (Koppenhofer and Kaya,1998; Koppenhofer *et al.,* 2002).

Factors affecting Interaction: Generalization on EPNs tolerance to insecticides cannot be given, because different results are related to nematode species and strain, chemical formulation, application dose and exposure time.

Differential reaction of EPNs with different pesticides: The species and strain of nematodes appear to be of key importance in determining its level of susceptibility to systemic insecticides (Koppenhöfer & Grewal, 2005; Atwa *et al.,* 2013). The only free-living stage is non-feeding third stage called the infective juvenile (IJ).Their mouth and anus are closed and thus the only point of access is the cuticle. Species that have the second stage cuticle fixed on the external surface of the third stage should be better protected. *H.bacteriophora* and *H.heliothidis* were less tolerant to some pesticides than *Steinernema* spp. The normal development of all the tested species was adversely affected after chemical treatment with most pesticides except for *S.glaseri*. This strain was the most tolerant to the toxic effects of pesticides organophophates and carbamates and appeared to be the most resistant and thus suitable for integration with pesticides (De Nardo and Grewal, 2003; Garcia-del-Pino and Jove, 2005;Laznik *et al*., 2012). The different effects between insecticides on survival of nematode IJs could be related to the different effects on nematodes chemical receptors and the respiratory metabolites.There was a low IJ mortality for *S. carpocapsae* when exposed to chlorpyrifos (Zimmerman and Crashaw 1990; Gutierrez *et al*., 2008). This insensitivity in the EPNs involves the presence of butyrylcholinesterase in the synapse of parasitic nematodes, protecting the acetylcholinesterase, and thus acting as a defense against such compounds (Selkirk *et al*., 2001). Mortality and infectivity of nematode may be related to the reduction in lipids in the EPNs after contact with insecticides (Wright and Perry 2002). Genetic selection can be used to enhance resistance of entomopathogenic nematodes to certain environmental stresses. Nematode resistance to some pesticides can be enhanced and thus oxamyl resistant strains of *H.bacteriophora* have been isolated. Reductions in nematode activity after exposure to chemicals are not accompanied with concomitant reductions in infectivity. Nematode exposed to these chemicals became quiescent but after being removed from contact with chemicals, became active again and are capable of infecting susceptible insect hosts.

The reason for the very slow death state may be due to rates of penetration, metabolism and detoxification of the chemical by the nematode (Forshler *et al.,* 1987). In addition to stressing the target hosts, infective juveniles can also be exposed to infectivity enhancing additives. Jaworska *et al*., (1996, 2002) demonstrated that manganese and magnesium cations enhanced *H. bacteriophora* infection in *G. mellonella* and *Sitona lineatus*. Knowledge of the potential reproduction loses attributable to the used pesticides will be help to calculate the required application rate of nematodes in the field. Endemic nematode strains may differ in sensitivity to different formulations of the same pesticide (Rovesti and Deseo, 1990; Grewal 2002). Therefore, before tank mixing newly isolated EPNs with any pesticides, their compatibility should be checked.

Chemical group of insecticide: The large variability between insecticides from the same chemical group in their compatibility with entomopathogenic nematodes make extrapolation of data between products unreliable (Rovesti and Deseo,1990), therefore each candidate product for an IPM system should be tested individually. Compatibility of EPNs and pesticides targeted only one specific group of pesticides, usually pesticides which are used against one specific pest, pesticides that belong to the same chemical group, e.g. carbamates, or have the same biological activity, e.g. nematicides. Some reports demonstrated that certain insecticides, particularly organophosphates and carbamates, possess nematicidal properties (Atwa, 1999). These insecticides induced adverse effects ranging from impaired movement, infectivity and reproduction to death of *Neoaplectana carpcapsae* IJs (Rovesti and Deseo,1990). Zimmerman and Cranshaw (1990) reported that carbaryl was significantly more toxic to *H. bacteriophora* (HP88 strain) than the *Neoaplectana* spp. after 24 h and 48 h of exposure to 1000 ppm, while *N. carpocapsae* and *N. bibionis* were not significantly affected by any of the concentrations tested. Zang *et al*., (1994) and Gordon *et al*., (1996) reported no toxic effects of several carbamates and minimal effects of a variety of organophosphates on nematode survival, infectivity and reproduction.

The infectivity of nematodes surviving an insecticide treatment was unimpaired after nematodes were freed from insecticides (Kaya and Burlando,1989). Several carbamates and organophosphates adversely affected the *in vitro* development and reproduction of *S. carpocapsae* (all strains), whereas this strain *S. carpocapsae* (all strains) was unaffected by the chlorinated hydrocarbon methoxychlor or the synthetic pyrethroid fenvalerate. García-del-Pino and Jové (2005) observed that *H. bacteriophora* and *S. carpocapsae* were similarly, highly tolerant to fipronil, whereas *S. arenarium* was more sensitive. However, fipronil concentration and exposure time affected badly the infectivity of Beninese EPN (Zadji, 2014). Chitin-inhibiting insecticides had been observed not affecting the viability of *Heterorhabditis bacteriophora* (Rovesti *et al*.,1988), *S. carpocapsae* and *Steinernema feltiae* .However, some pesticides can reduce nematode survival and infectivity (Grewal *et al*., 1998). Some chemicals used as inert ingredients or adjuvants in formulations can be toxic to nematodes hence compatibility of each formulation with the specific nematode species should be evaluated.

Exposure time: Entomopathogenic nematodes are reported to be tolerant to short exposure (2-6h) to most agrochemicals,

Table 1. Compatibility of Entomopathogenic nematodes with chemical pesticides

Nematode	Insecticide	Compitibility	Test Insect	Interaction	Reference
Steinernema carpocapsae DD 136	diazinon		*Hylemia* spp	as effectively as the chemical insecticide	Cheng &Bucher , 1972
Neoaplectana dutkyi DD-136	Organophosphates, formothion , phosalon	toxic			Rao *et al*., 1975
S.carpocapsae *S. feltiae*	Oxamyl Organophosphate and carbamate	Non-toxic toxic			Fedorko *et al*.,1977a; Fedorko *et al*.,1977b
N. carpocapsae	Organophosphates and carbamates	toxic			Hara & Kaya, 1982
N.carpocapsae	Organophosphates and carbamates		beet armyworm	toxic	Hara & Kaya,1983a Hara & Kaya,1983b
S. feltiae DD-136	carbaryl, dimethoate, endosulfan, malathion	Non- toxic			Das & Divakar, 1987
Heterorhabditis sp.	chlorpyrifos, endosulfan		*Otiorhynchus sulcatus*	slightly toxic	Heungens & Buysse, 1987
N.carpocapsae	Hostathion[triazophos]		*Agrotis ipsilon*	100% mortality	El-Kifl& Sammour, 1988.
H. bacteriophora	parathion, phorate, terbufos, fonofos, isofenphos, phoxim, aldicarb, carbofuran, methomyl, metham sodium [metham] ,phenamiphos [fenamiphos] Aldicarb, carbofuran, methomyl	toxic	*G.mellonella*	antagonistic	Rovesti *et al.,*1988
S.feltiae	fenamiphos18 mg a.i./kg dry sand		*G.mellonella*	76.7% mortality	Kaya &Burlando,1989
N. carpocapsae	chlordecone		*Cosmopolites sordidus*	Synergistic	Kermarrec & Mauleon, 1989
Steinernema sp., *Heterorhabditis* spp *S. carpocapsae* *S. feltiae H. bacteriophora,* *H. heliothidis, S.carpocapsae* *S. feltiae*	parathion, aldicarb, methomyl, flubenzimine, metham sodium and fenamiphos phosphamidon, diazinon, chlorpyrifos, endosulfan	toxic Non toxic	*G.mellonella*	Negligible effect	Rovesti 1989; Rovesti & Deseo, 1990; Rovesti *et al*.,1990; Rovesti &Deseo , 1991
H. sp. HP-88 *N. carpocapsae* *N. bibionis*	Carbaryl,bendiocarb, Diazinon Carbaryl,bendiocarb, chlorpyrifos	Highly toxic Less toxic Highly toxic			Zimmerman & Cranshaw, 1990
S. feltiae	Bentazone, ioxynil, hexaconazole, cyromazine, buprofezin quizalofop-ethyl, tralkoxydim, sulfur ,potassium soap isofenphos,permethrin,fluazifop butyl, iprodione	nontoxic toxic	*Tenebrio molitor*	synergistic	Vainio &Hokkanen, 1990; Vainio, 1994
S.carpocapsae *H.bacteriophora*	oxamyl		*Galleria mellonella*	antagonistic	Gaugler& Campbell, 1991
H. bacteriophora H. heliothidis *S.glaseri*	organophosphates and carbamates	less tolerant toxic	*Cosmopolites sordidus*		Sirjusingh *et al*.,1991
S.kushidai	diazinon ,fenthion	toxic	*Anomala cuprea*		Fujiie *et al.,*1993
S.carpocapsae All strain	acephate, permethrin		*Spodoptera litura*	synergistic	Ishibashi& Takii ,1993
S.carpocapsae	Cartap, profenofos, pyraclofos diazinon, dichlorvos, fenthion, malathion, trichlorfon, propetamphos,prothiofos cartap, profenofos	toxic weak toxicity	*Spodoptera litura*	Antagonistic	Zhang *et al*.,1994

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S.carpocapsae S. feltiae	oxamyl , fenamiphos	Non toxic	*G. mellonella*	Reduced infectivity	Patel &Wright, 1996
S.carpocapsae All strain *S.feltiae* Umea strain	Fenoxycarb, carbofuran	toxic			Gordon et al.,1996
H.bacteriophora	malathion	toxic	*Spodoptera litura*	80% mortality	Baweja &Sehgal, 1997
H.bacteriophora strain HP88	fenamiphos, oxamyl, avermectin	Less toxic			Glazer *et al*.,1997
S. feltiae	Formalin , chlorine	highly toxic			Grewal *et al.*, 1998
H. bacteriophora	imidacloprid	Non toxic	white grubs *Cyclocephala hirta* *C. pasadenae*	synergistic	Koppenhofer &Kaya, 1998
S.carpocapsae H.bacteriophora	terbufos, fonofos, tefluthrin		*Diabrotica virgifera virgifera*	Additive ,synergistic	Nishimatsu & Jackson, 1998
S.carpocapsae	Phosphamidon, Monocrotophos Phorate	Less toxic	*Corcyra cephlonica*	Synergistic	Gupta, &Siddiqui, 1999
S.glaseri, S. feltiae *H. megidis*	Carbosulfan, carbofuran	Non toxic	*Galleria mellonella*	synergistic	Bednarek *et al*., 2000
Steinernema feltiae	Trichlorfon , dimethoate		G. mellonella *Liriomyza huidobrensis*	Synergistic	Head *et al.,* 2000
S.glaseri H.bacteriophora *S. kushidai*	imidacloprid	Non toxic toxic	white grubs	Synergistic antagonistic	Koppenhofer *et al.,*2000
H.marelatus IN Strain	halofenozide	Non toxic	*Popillia japonica*	No synergistic effect	Mannion *et al.*,2000
H.indica S.sp.	fenvalerate, endosulfan Quinalphos	Non toxic toxic	*Galleria mellonella*	antagonistic	Hussaini *et al.,*2001a
S.bicornutum *H. indica*	malathion, endosulfan, carbofuran, quinalphos, fenvalerate	Non toxic	*Galleria mellonella*	no additive or synergistic response	Hussaini *et al*. 2001b
H.bacteriophora EBN10k *Steinernema* sp. EBN1e	chlorfluazuron, thiocyclam and benomyl Methomyl, Benomyl, Trimiltox forte Diafenthiuron Chlorfluazuron		*Spodoptera littoralis*	81.5 % mortality	Atwa, 1999; Atwa *et al*.,2013; Atwa, 2014
S.feltiae	chlorpyrifos			antagonistic	Chen *et al*.,2003
S.feltiae	diflubenzuron (Adept IGR), acephate (Orthene), fenoxycarb (Precision 25WP)	Non toxic	*Galleria mellonella*	Increased infectivity	De Nardo& Grewal, 2003
H.bacteriophora HP88 *S. carpocapsae* All strain	Thiamethoxam, trichlorfon halofenozide, aluminum tris, trichlorfon, and carbaryl Imidacloprid aluminum tris , trichlorfon	toxic	*Galleria mellonella*	antagonistic synergistic antagonistic	Alfred &Grewal, 2004
S.feltiae *H. bacteriophora*	Imidaclopride, Fipronil , Chlorpyriphos	Non toxic			Peters& Poullot, 2004
H. megidis S.*feltiae* *S. glaseri*	carbosulfan , carbofuran		cockchafer's grubs	synergistic	Bednarek *et al* .,2004
S.scapterisci	acephate, bifenthrin, and imidacloprid	nontoxic	*Scapteriscus vicinus*	Synergistic upto40% mortality	Barbara &Buss, 2005
S.carpocapsae *H. bacteriophora* *S. arenarium*	fipronil	Non toxic toxic		negligible effects on the infectivity	García- del-Pino& Jové, 2005.

S.carpocapsae	thiacloprid,		*Galleria mellonella Bemisia tabaci*	synergistic	Andrew *et al*.,2008
S. carpocapsae	thiacloprid and spiromesifen		*Bemisia tabaci*	synergistic	Cuthbertson *et al*.,2008
S.feltiae Rioja (native) ENTONEM® (commercial)	chlorpyrifos , pirimicarb cypermethrin	Non toxic	*Spodoptera littoralis*	Reduce virulence and reproductive potential	Gutiérrez *et al*.,2008
H.bacteriophora	chlorantraniliprole	Non toxic	white grubs		Koppenhöfer &Fuzy, 2008
H.bacteriophora	Carbofuran, Carbosufan, imidaclopid	Non toxic			Priya & Subramanian ,2008
S. glaseri	Carbofuran, Carbosufan, Imidaclopid Phorate dimethoate	nontoxic			
S.carpocapsae	imidacloprid		*Rhynchophorus ferrugineus*	synergistic	Dembilio *et al*.,2010
S.feltiae, S. arenarium S. kraussei	clopyralid, fluoroxypyr, , sodium2-methoxy-5-nitrophenol, propamocarb, , fenithrothion , propargite oxamyl, sulphur, trifluralin, chlorpyrifos, lambda-cyhalotrin	Non toxic toxic			Nermut &Mracek, 2010.
S. feltiae(B30) Entonem	Thiametoxam		*Leptinotarsa decemlineata*		Laznik *et al*.,2010
H.indica, *S.carpocapsae S. glaseri*	Lorsban^{TM} (chlorpyrifos), Decis^{TM} (deltamethrin), Match^{TM} (lufenuron), Deltaphos^{TM} (deltramethrin+triazophos), Dimilin^{TM} (diflubenzuron), Stallion^{TM} (gamacyhalothrin) Karate Zeon^{TM} (lambdacyhalothrin) Vexter^{TM} (chlorpyrifos), Galgotrin^{TM} (cypermethrin), Certero^{TM} (triflumuron), Talcord^{TM} (permethrin)		*Spodoptera frugiperda*	compatible	Negrisoli *et al*.,2010
S. feltiae	kinoprene, lufenuron, methomyl, metoxyfenozide, oxamyl, piperonyl-butoxide, pyriproxyfen, tebufenozide	Non toxic			Radová ,2010
S.carpocapsae H. indica (Meghalaya isolates)	Monocrotophos, Dicofol ,	Non toxic			Devi, 2011
S.feltiae	thiamethoxam		*Trialeurodes vaporariorum*	No efficacy in combination	Laznik *et al.,*2011
S.feltiae H.bacteriophora	Fenpyroximate, tebufenpyrad		*T. molitor*	antagonistic	Radová, 2011
S.masoodi, S. seemae, S. carpocapsae S. mushtaqi	Endosulfan , Monocrotophos		*Corcyra cephalonica*	Less infectivity	Pervez &Ali, 2012
S.asiaticum H.bacteriophora	Endosulfan Malathion	Non toxic	*Plutella xylostella*	synergistic	Kumar *et al.,*2013
S.carpocapsae	Imidacloprid, Thiomethoxam	Non toxic	*Galleria mellonella*	synergistic	Kulkarni *et al*.,2013.
S. carpocapsae S. kraussei Steinernema feltiae H. bacteriophora	imidacloprid	Non toxic			Laznik &Trdan ,2013
H.zealandica S.yirgalemense	Cyperfos 500 EC®, Cryptogran™, Helicovir™, Nu-Film-P® and Zeba®,	Non toxic	*Tenebrio molitor*	synergistic	Van Niekerk & Malan, 2014
S.abbasi	Profenophos , Lambda-cyhalothrin, Dimethoate, Quinalphos Chlorfenapyr, Chlorantranilprole, Bifenthrin Dichlorvos	Non toxic toxic	*Galleria mellonella*	synergistic	Kumar *et al.,*2015
H. bacteriophora S.feltiae	imidacloprid	Non toxic			Le Vieux & Malan, 2015
S.thermophilum	triazophos, chlorpyrifos and endosulfan	toxic	*Galleria mellonella*	antagonistic	Anes & Ganguly, 2016
S. carpocapsae H. indica			*Helicoverpa armigera*	Moderate effect	Devindrappa *et al*.,2017
H.amazonensis GL *H.amazonensis* MC01	Avicta 500 FS®, Maxim®, Cruiser 350 FS®, Fortenza 600 FS®, Amulet®	Non toxic toxic	*Tenebrio molitor*	Reduced infectivity	Magnabosco *et al.,*2019

including insecticides, acaricides, fungicides and herbicides (Rovesti and Deseo 1990) and therefore, can often be tank-mixed. However, long exposure to some plant protection products can affect the efficiency and reproduction of the nematodes (Negrisoli *et al*., 2010). Atwa (1999) observed that length of exposure to the insecticides had little discernible effect on nematode survival and reproduction but depended on insecticidal concentration.

Temperature: Temperature influenced IJ mortality when the nematodes were mixed with insecticides. It is a known fact that between 20 and 26°C, the activity of EPNs is the highest and that we can relate their sensitivity to insecticides with their ability to withstand osmotic stresses.

Time of application: For the development of a successful IPM system, simultaneous use of insecticides and biocontrol agents may be required. Effective field control of lepidopteran larval pests has been reported following mixed applications of *S.carpocapsae* with chemical insecticides, and the study suggested that simultaneous use would synergistically improve insect control (Ishibashi, 1992). However a limited range of insecticides can be applied simultaneously with *S.feltiae* (Head *et al*., 2000). IPM approaches may require sequential rather than simultaneous application of chemical insecticides and entomopathogenic nematodes. Sequential treatments offer a greater flexibility in timing applications of the different control agents, many of which are known to cause differential mortality to the various life stages of the target pests (Williams and Walters, 1994).Thus targeting of a particular life stage with the most appropriate control measure remains a viable option. High level of control of leafminer larvae can be achieved by the application of *S.feltiae* to vegetable foliage previously treated with insecticides (Head *et al*.,2000). The addition of small amount of certain insecticides causes physiological weakening of the insect organism and reducing its resistance to EPNs. Investigation is necessary to determine whether prior application of sublethal doses of insecticides facilitates nematode invasion and whether prior exposure to nematodes lowers insect resistance to insecticides. One way of using the incompatible nematodes and insecticides would be applying them at different time after the period of persistence of the product, or vice versa (Negrisoli *et al*., 2010). Imidacloprid disrupts a grub's normal nerve function, which drastically reduces its activity, affects grooming and evasive behaviors, and facilitates nematode attachment onto the cuticle. Thus Imidacloprid is synergistic with *S. glaseri* (Steiner) or *Heterorhabditis bacteriophora* Poinar against white grubs (Koppenhöfer *et al*., 2000a, 2000b). Mole crickets treated with imidacloprid survived longer than those treated with the other insecticides, but still died from nematode infection, Pesticides which increase mole cricket activity, rather than slow it down, may result in increased contact with ambusher nematodes , *S.scapterisci*.

Application rate: It is necessary to calculate the application rate of the nematodes based on knowledge about the potential efficacy losses due to certain pesticides. Entomopathogenic nematodes are relatively resistant to many pesticides in recommended dosage, besides showing synergy between EPNs and chemicals insecticides.

Conclusion

Entomopathogenic nematodes (EPNs) could be effective in integrated pest management (IPM) and sustainable programs as long-term suppressive agents used in combination with commercially available insecticides. Due to the continuous introduction of new molecules or active ingredients and formulations in different market segments and to differences in susceptibility of nematode species/strains to pesticide formulations, it is difficult to provide up to date information for each of the chemical pesticides. The observed results for the compatibility/interaction effects of insecticides on nematodes not only make application of nematodes in agro-ecosystems easier, but also promising their use in integrated pest management systems.

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