Efficacy Patterns of Biopesticides Used in Potting Media

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ABSTRACT Greenhouse nursery production represents a large and growing industry. In recent years there has been a shift both toward the use of soilless media and integrated pest management. Application of entomopathogens such as nematodes, fungi, and bacteria against soil-dwelling insect pests is a sustainable alternative to insecticide centered control programs. Soilless media (peat, coir, or bark-based) are designed to provide a plant pathogen free growing environment. The potential impact of soilless media on the efficacy of entomopathogens is often overlooked. In this review, we summarize the findings of research studies in which entomopathogens were applied to control insect larvae or eggs in soilless media. Most studies concentrated on controlling coleopteran larvae, primarily *Otiorhynchus sulcatus*, and dipteran larvae in the *Bradysia* genus. Entomopathogenic nematodes appear to perform better in peat–based media, but no clear trend emerged for entomopathogenic fungi. Control of *Bradysia* spp. was usually around 50% but nematodes fared better in bark–based media. The effect of media may not be as important for fungi and bacteria because they are non-motile organisms, whereas nematodes move along the water film to find the host. However, application method may influence the efficacy of fungi and bacteria. In general, few conclusions can be drawn about the direct effect of media on biological agents and we suggest that detailed information on the physical properties of soilless media be provide in future studies.

KEY WORDS : Entomopathogens, greenhouse insect pests, soilless media

INTRODUCTION

Biopesticides currently are integrated into many diverse agricultural production schemes. These materials can be effective and safe, but their use requires more sophistication than chemical pesticides on the part of the user. Many of these products have specific requirements for storage and application, and to treat them like a chemical pesticide often results in failure. As biological organisms they require appropriate biotic as well as abiotic conditions for success. Users would benefit from learning how to maximize efficacy before or after the organisms have been applied. Environmental manipulation has been suggested, but in most crop situations, the large scale is an insurmountable barrier. Altering the humidity or soil type of a cotton field, say, is not possible. However, greenhouse production of potted plants offers a system where these types of alterations are possible and fairly tractable.

The greenhouse industry is one of the fastest growing agricultural markets in North America, worth $16.9 billion in 2006 (Jerado, 2007). Greenhouses provide a controlled growing environment that can be altered to accommodate the requirements of many plant species. Unfortunately, ideal plant production conditions often favor development of numerous plant pests, including insects. Soil-borne insects

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damage roots by feeding on root hairs, young roots, and feeding can girdle older roots, all of which reduce growth and vigor of aboveground plant structures, limit water and nutrient uptake and in severe infestations can kill the plant. Spurred partially by insecticide-use restrictions, nursery management practices now integrate biological control methods for pests, such as entomopathogens, into production plans for greenhouse and nursery crops (Tomalak *et al.*, 2005). More than 30 biological control products are commercially available for use including ones based on entomopathogenic nematodes, entomopathogenic fungi and bacteria, parasitoids, predatory insects, and predatory mites (Tomalak *et al.*, 2005). Entomopathogenic nematodes and fungi are often used to control soil-dwelling insect pests such as the black vine weevil (*Otiorhynchus sulcatus* (Coleoptera: Curculionidae)), fungus gnats/mushroom flies/shore flies (*Bradysia spp*. and *Scatella spp*. (Diptera: Sciaridae)), scarab larvae (Coleoptera: Scarabidae), citrus root weevil (*Diaprepes abbreviatus* (Coleoptera: Curculionidae)), and Western flower thrips (*Frankliniella occidentalis* (Thysanoptera: Thripidae)). Because of their cryptic behaviors, populations can be difficult to monitor. Luckily, many of the conditions that benefit pests, such as warm temperatures and high soil moisture, also benefit control efforts using entomopathogens (1991). While most entomopathogens do best under high moisture conditions, soil texture and particle size can limit efficacy or persistence. For instance, in high clay soils, the movement of entomopathogenic nematodes is restricted due to the small particle size (Barbercheck, 1992). Fine silt soils can also retard movement.

PHYSICAL PROPERTIES OF SOIL

The physical composition of the environment in mineral soils has been shown to influence efficacy. We use the term mineral soils to encompass all natural or field soils comprised of varying amounts of silt, sand, and clay. Efficacy in mineral soils has been linked to physical characteristics of the soil environment, especially pH, moisture, particle size, and the interaction of the entomopathogen and the target insect (Villanii and Wright, 1990; Barbercheck, 1992). Interspecific differences of movement, dispersal, and efficacy exist between nematode species that can be linked to soil characteristics. Soil pH and temperature impact efficacy of nematodes. Acidic soils appear to limit efficacy of entomopathogenic nematodes (Barbercheck, 1992), whereas the reverse is true for fungi and bacteria (Quesada-Moraga *et al.*, 2007), although in soilless media, amendments such as dolomite lime or calcium carbonate lime are added to adjust pH, especially in peat-based media. *Steinernema* spp. have lower temperature threshold and wider range (3–14°C) than most *Heterorhabditis* spp. which can survive at warmer thresholds (10–16°C) (Barbercheck, 1992). Temperature fluctuations can be rapid in container plants, and most greenhouse production functions at high temperatures which may provide a physiological advantage for *Heterorhabditis* sp. in some areas. Sporulation of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) occurs from 10–35°C (Barbercheck, 1992). Temperature can significantly affect efficacy of *B. bassiana*, with a higher infection rate at 16–24°C than at 8°C (Studdert and Kaya, 1990). However, the impact of this in greenhouse production may be negligible because of the high temperature conditions typical of greenhouses.

Soil moisture is very important for entomopathogenic nematodes and is often related to particle size. The water available to plants and animals is often measured by water potential in negative kilopascales (–kPa), the amount of energy required to remove water for the soil. Decreasing water potential is correlated with decreasing thickness of the water film surrounding soil particles. As water potential decreases, the water is drained from the soil beginning with large soil particles. The smallest pore space has a very negative water potential and water that is unavailable to plants (Barbercheck, 1992). EPN efficacy is reduced in soils with either inadequate (–1000 kPa) or excess moisture (–1 kPa) (Wallace, 1958; Koppenhöfer and Fuzy, 2007), however there are species-specific differences. In sandy loam soil, establishment of *H. bacteriophora* was significantly less at –1000 and –3000 kPa, for *H. zealandica* and *S. glaseri* establishment was significantly greater at –10 kPa (Koppenhöfer and Fuzy, 2007). As the water potential decreases, nematode movement becomes more difficult because the thickness of the water film is diminished. The relationship between water potential and entomopathogenic fungi and bacteria is not as clearly defined. In moderately dry soils, *B. bassiana* had significantly higher infectivity at –37 kPa and –200 kPa but this only occurred at a high concentration (3.2×10^7 conida/cm^3) (Studdert and Kaya, 1990).

The speed at which water drains (or evaporates) from the soil depends on the relative proportions of particle sizes in soil; sand (0.05–2.00 mm), silt (0.002–0.05 mm) and clay (< 0.002 mm). Generally, efficacy is highest in sandy or sandy loam soils and decreases with finer textured soils (with higher clay content) due to physical characteristics of the soils (Molyneux and Bedding, 1984; Kung *et al.*, 1990). Large pore sizes found in sandy loam and sand-based organic soils (40% OM) permitted the greatest amount of movement of *S. carpocapsae* and *H. bacteriophora* (Barbercheck and Kaya, 1991); however, efficacy is species-specific. Low efficacy was achieved with *H. baujardi* against *Conotrachelus psidii* (Coleoptera: Curculionidae) in sandy soil (58%) (Del Valle *et al.*, 2008). On the other end of the soil spectrum, *H. bacteriophora*, *S. carpocapsae*, and *S. glaseri* survival decreases in finer textured soils and low efficacy is related by increasing percentages of clay particles (Georgis and Poinar, 1983; Molyneux and Bedding, 1984; Kung *et al.*, 1990; Barbercheck and Kaya, 1991). Shapiro and McCoy (2000) found that *H. bacteriophora* and *S. riobrave* has higher efficacy in soil containing 80% silt (4.1% OM) and slow water release curve. However, small pore sizes and poor aeration results in high moisture potential which can lead to an anaerobic soil environment which is a fitness cost to the nematodes (Kung *et al.*, 1990). In fine textured soils, infectivity for *S. carpocapsae*, *S. glaseri* and *H. bacteriophora* were reduced (Koppenhöfer and Fuzy, 2006); however, this result is variable among nematode species. Soilless media tend to be high in organic matter because their primary materials are peat moss, bark or sawdust. We hypothesize that these materials will also have large pore sizes and, if watered correctly, should provide a near ideal environment for EPNs.

The relationship between soil particle size and efficacy of fungi and bacteria is not as clear, especially for clay content (Barbercheck, 1992; Quesada-Moraga *et al.*, 2007). In media with high clay content, Fuza and Richter (2004) were able to significantly reduce populations of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), achieving greater than 30% infection 10 weeks post application when applying *B. bassiana*. However, only 1.7% infection was obtained in media with high clay content. In another study using *B. bassiana* against the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) in a soil-based media with a high sand content, Ekesi *et al.* (2003) attained 80–90% efficacy with four isolates of *M. anisopliae*. Field soils with higher than 3% organic matter was more likely to have endemic populations of entomopathogenic fungi or bacteria (Quesada-Moraga *et al.*, 2007).

The propensity of entomopathogenic fungi and bacteria to germinate/proliferate in soil may influence both their efficacy (important for curative treatments) and persistence (crucial for prophylactic treatments). Premature germination in the absence of hosts may reduce control levels (Li and Holdom, 1995). While the performance and efficacy on entomopathogenic fungi and bacteria may be related to the abiotic/physical properties of the soil, there is evidence that both soil amendment and sterilization can alter efficacy and persistence though effects on available nutrients (West *et al.*, 1985; Li and Holdom, 1995) and fungistatic properties of soil (Lingg and Donaldson, 1981) of soil. The same requirements for efficacy in soil should hypothetically be true for soilless media as well, but will be influenced by the composition of the soilless media. While prior sterilization of media could conceivably affect EPN performance, the primary factors that we hypothesize to impact efficacy of EPNs are water potential and pore size. Both affect the water film which the nematodes move through to find their host.

SOILLESS MEDIA

In recent years, nursery production has transitioned from the use of mineral soil-based potting media to soilless culture (Johnson, 1985). Soilless culture includes hydroponic systems and solid media systems called soilless media: they are made of simple or complex mixtures of materials. The combination of these materials is what makes them attractive for use in greenhouse settings, where the environment can be manipulated. Most commonly, soilless media are composite mixes composed of shredded Sphagnum peat, shredded coir, composted bark or sawdust-based materials with the addition of sand, vermiculite, and/or perlite. Less commonly used growing media contain bagasse or rice hulls but both have low water-holding capacity and are not used alone. Peat-based mixes were adopted by the greenhouse industry in the 1960's and are often termed 'peat-lite' and are composed of peat mixed with either vermiculite or perlite to increase water retention and porosity (Boodley and Sheldrake, 1973; Ingram *et al.*, 1991). Vermiculite is a micaceous material that can hold and release large quantities of water and minerals. Perlite is a volcanic rock that, unlike vermiculite, does not have any cation exchange capacity and is used primarily because it can hold moisture. Sand is often added because it can provide structural support, weight, and water drainage. Coconut coir, is the ground up mesocarp of coconut (*Cocos nucifera* L.) and has been used as a sustainable substitute for peat, a limited resource, because it has similar physical characteristics (Olson *et al.*, 2002). There is an indication that survivorship of some insects, such as fungus gnats, is increased with the use of coarse-textured peat-based media (Olson *et al.*, 2002). Ideally, these manufactured soilless mixes provide a pathogen-free physical support system necessary for plant growth, and thus avoid some of the major problems that are associated with mineral soils. The available nutrients, percent organic matter, pH, and water holding capacities (pore size) of soilless media vary greatly from each other and from mineral or composite soils. The structural and chemical aspects of these media can be manipulated by the grower, or manufacturer, so soilless media containing peat, coir, or bark have greater potential for nutrient and water retention than do most mineral soils (Johnson, 1985). On the downside, soilless media do not have all the available nutrients and micronutrients present in mineral or even composite soils, so most plant species grown in them require significant nutrient input. Additional inputs for container plant production include various pest management materials.

Soilless media were not commonly used in research studies prior to the 1970's and research that specifically examined the influence of soilless media on entomopathogens did not gain momentum until the turn of the century. This body of research primarily uses soilless media in studies that evaluate either persistence or efficacy of a biological control agent. Few of these studies focus on the importance of soilless media type on these factors; indeed there is often no available information on what medium is used in greenhouse efficacy trials.

While use of biopesticides is increasing due to insecticide restrictions and public interest, our goal is to focus on the persistence and efficacy of entomopathogenic nematodes, fungi, and bacteria used to control soil dwelling herbivores in a containerized greenhouse or nursery setting. We also consider the influence of associated factors such as the target pest and mode of application, which may modify the importance of the soilless media on entomopathogen performance. Our objective is to synthesize some new conclusions based on these studies and pose reasons underlying the efficacy of entomopathogens in the different soilless media. Additionally, we can make some recommendations for matching media with biopesticides that optimize efficacy.

A keyword search of electronic databases yielded a total of 26 relevant studies. Studies were generally limited to those for which data on persistence and efficacy were available, though a few exceptions were made to broaden the taxonomic representation of biological control agents. Treatments were classified as being applied to either i) peat or coir-based (including loam-based composts) ii) peat:sand based and iii) bark based media. Here we summarize the mean efficacy data from 26 publications and 263 bioassays that test entomopathogen efficacy against the soil dwelling stage in soilless media by entomopathogen species and insect order (Table 1). Percent mortality were calculated from NIH Image J (downloaded from http://rsb.info.nih.gov/ij) and where needed, data were corrected for control mortality using Abbott's formula (Abbott, 1925).

ENTOMOPATHOGENIC FUNGI AND BACTERIA

Products based on entomopathogenic fungi and bacteria are treated separately from the nematodes because while nematodes move through the medium, these products do not; thus, their requirements are different. Thus, they must rely on their mobile hosts coming in contact with them. Fungal spores and hyphae are able to penetrate the host cuticle, while most bacteria must be ingested. Once inside the host, proliferation of the fungi or bacteria generally results in host death (though in some cases the host may experience sub-lethal effects of infection). Here we focus on the persistence and efficacy of entomopathogenic fungi and bacteria used to control root herbivores in a containerized nursery setting. We also consider the influence of associated factors such as the target pest and mode of application, which may modify the importance of the soilless media on entomopathogen performance. Here we summarize a total of 13 studies, comprising a total of 149 entomopathogen applications. Due the paucity of suitable data for other entomopathogens, it will be focused primarily on studies of *B. bassiana* and *M. anisopliae*.

Peat/Loam-Based

We identified three studies in which entomopathogens were used against pests infesting a peat/loam soil. In a loamy-soil mixed with peat, (Kowalska, 2008) treatment with *B. brongniartii* and two Neem formulations respectively provided 87 and 92% control of the black vine weevil, *O. sulcatus*. However, efficacy levels were variable in two other studies targeting *Delia* spp. (Diptera: Anthomyiidae) in a similar potting medium. Chandler and Davidson (2005), using two isolates of *M. anisopliae* against *D. radicum* feeding on *Brassica olearcea* found that when the isolates were pre-mixed into the media, the treatment was completely ineffective. However, when applied as an aqueous suspension, efficacy levels of 50% and 90% were obtained for the two isolates (Chandler and Davidson, 2005). A third study examining the potential of several *B. bassiana* and *M. anisopliae* isolates to control *D. radicum* and *D. floralis* failed to obtain any significant control. In this case, the treatment was applied as an aqueous suspension (Vanninen *et al.*, 1999).

Peat or Coir-Based

The studies of the use of entomopathogens in peat or coir-based media comprise the bulk of the review. From ten studies we obtained 90 data points for comparison. The efficacy achieved ranged from 2.3 to 100%. Targeted pests included western flower thrips, black vine weevil, and *Scatella stagnalis*. Stanghellini and El-Hamalawi (2005) were able to achieve 97 and 100% control of shore flies *S. stagnalis* in two experiments using *B. bassiana*. A unique feature of this study is that the material was applied as a formulation of colonized millet seed.

Ansari *et al.* (2008) evaluated the ability of several strains of *B. bassiana* and *Metarhizium* sp. to control western flower thrips in coir, peat, and peat amended with organic matter (10 or 20% composted green waste). In a laboratory screening trial, *B. bassiana*, applied as a pre-mix provided significant levels of control ranging from 51 to 79%. In the same trial control with strains of *Metarhizium* sp. ranged from 47% to 94%. Across species and strains, after applying Abbot's correction, an apparent trend emerges for efficacy to decrease with increasing organic matter amendment of the peat-based media. However, we cannot say much about the possible effect of organic matter because this trend appears to be primarily attributable to increased thrips mortality in the controls amended with composted green waste. The effect of application method, (aqueous drench or pre-mix) on thrips suppression by *M. anisopliae* was assessed in a second greenhouse trial, but no difference in control levels were evident.

Table 1: Mean percent mortality of entomopathogens in soilless media against greenhouse insect pests summarized from 26 research manuscripts.

Pathogen Species	Material	Mean Percent Mortality (Range) Against Target Insect Order[1] Coleoptera	Diptera	Lepidoptera	Thysanoptera	Hymenoptera	Total # Evaluations
Heterohabditis heliothidis	Peat/Sand	82 (64–90)					4
Heterohabditis marelatus	Peat/Sand	100 (100)					4
	Peat	98 (93–100)	0 (0)				5
	Bark		0 (0)				1
Heterohabditis zealandica	Peat/Sand	83 (83)					1
	Peat		2 (2)				1
	Bark/Peat/Sand	44 (83)					1
	Bark		0 (0)				1
Heterohabditis bacteriophora	Sand	45 (18–68)					16
	Mineral	36 (28–49)					3
	Peat	98 (92–100)	0 (0)				5
	Peat/Bark/Clay				54 (29–70)		6
	Bark/Peat/Sand	61					1
	Bark		47 (47)				1
Heterohabditis baujardi	Mineral	32 (6–50)					4
Heterohabditis indica	Peat		55 (55)				1
	Bark		51 (51)				1
Steinernema carpocapsae	Peat		0 (0)				1
	Bark/Coir		43 (5–84)				4
	Peat/Bark/Clay				50 (39–60)		3
	Bark		0 (0)				1
Steinernema diaprepes	Peat/Sand	93 (93)					1
Steinernema feltiae	Sand			14 (13–14)			2
	Peat/Sand	75 (71–78)					4
	Peat		37 (14–73)				4
	Bark/Coir		35 (0–56)				5
	Peat/Bark/Clay				48 (12–69)		9
	Peat/Bark		62 (61–63)				2
	Bark		17 (0–34)	100 (100)			4
Steinernema glaseri	Sand			87 (73–100)			2
	Bark/Peat/Sand	57 (57)					1

Pathogen Species	Material	Mean Percent Mortality (Range) Against Target Insect Order[1]					Total # Eval-uations
		Coleoptera	Diptera	Lepidoptera	Thysanoptera	Hymenoptera	
	Bark			7 (0–13)			2
Steinernema riobravae	Sand	88 (70–100)					16
	Mineral	77 (43–95)					6
	Peat/Sand	88 (88)					1
	Peat	61 (39–75)					4
Xenorhabdus nematophilus	Peat	96 (92–100)		29 (26–33)			4
Metarhizium anisopliae [2]	Mineral		83 (80–90)				4
	Peat/Loam		20 (0–89)				7
	Peat	64 (2–100)			75 (49–94)		47
	Coir	91 (87–96)			77 (64–94)		12
	Bark	71 (30–100)			77 (46–96)		16
	Perlite	86 (81–91)					2
Metarhizium flavoviridae	Peat				51 (46–56)		3
	Coir				51		1
	Bark				44		1
Photorhabdus fumosoroseus	Peat/Loam		0 (0)				1
	Peat				61 (57–67)		6
	Coir				64 (58–70)		2
	Bark				60 (57–63)		2
Beauveria bassiana	Mineral					24 (2–37)	3
	Peat/Loam		0 (0)				4
	Peat		99 (97–100)		60 (51–79)		8
	Coir				63 (53–73)		2
	Bark				60 (46–73)		2
Beauveria brongniartii	Peat/Loam	87 (87)					1
Bacillus thuringiensis subsp. *israelensis*	Bark/Coir		8 (0–16)				2
	Peat/Bark		5 (1–9)				2
Neem	Peat/Loam	92 (92)					2
	Peat	40 (40)					1

[1] Mortality was assessed at the end of the evaluation period of each experiment.
[2] One of the studies summarized here evaluated *M. anisopliae* efficacy against Coleopteran eggs in soil.

We identified six studies that tested entomopathogens against black vine weevil in peat and coir based growing media. Moorhouse *et al.* (1993a) showed greater than 96% control of weevil eggs with three *M. anisopliae* isolates applied 20 weeks prior to the introduction of the eggs. In the same study, the authors did not find a significant difference in *O. sulcatus* egg mortality between drench and spore incorporation application methods. In a separate experiment with three strains of *M. anisopliae*, Moorhouse *et al.* (1993b) achieved greater than 60% control of *O. sulcatus* eggs placed in soil to which conidia had been applied in an aqueous drench 16 weeks earlier.

Shah *et al.* (2007) looked at the ability of *M. anisopliae* to control *O. sulcatus* eggs in coir, peat, and peat-based media amended with composted green waste. They also asked whether efficacy was influenced by application method (homogeneous incorporation or aqueous soil drench). Although there wasn't much of a difference in coir, in each of the three peat-based media the aqueous drench was about 25% more effective than homogenous incorporation of conidia. Levels of control for both application methods were lower in the 100% peat media than in media amended with composted green waste. Another study by Shah *et al.* (2008) assessed whether Neem might act synergistically to enhance the efficacy of *M. anisopliae*. The authors tested single and combined treatments of *M. anisopliae* and Neem cake against black vine weevil egg and larval stages in a peat based medium. The highest *M. anisopliae* dosage resulted in 51 and 60% control of eggs and larvae respectively, although the authors note that all recovered larvae eventually showed signs of *M. anisopliae* infection. Neem when applied alone, led to 98 and 40% control of weevil eggs and larvae, respectively; while the combined fungus/Neem treatment generated 100% control of eggs and 95% control of larvae. When using late instar *O. sulcatus* to repeatedly bait soil premixed with *M. anisopliae* for 133 DAT, Bruck (2006) obtained between 86 and 94% weevil mortality in two experiments using coir and peat media. Having determined that *M. anisopliae* is able to colonize the roots of *Picea abies*, Bruck investigated whether the presence of the host plant would influence entomopathogen performance. However, no effect of host plant presence was detected.

Though normally associated with their symbiotic nematode hosts, Mahar *et al.* (2008) studied the ability of *Xenorhabdus nematophilus* cell suspensions and filtrates to kill *O. sulcatus* and *G. mellonela* larvae. Against black vine weevil, cellular suspensions and cell-free filtrates provided 92 and 100% control, respectively. Mortality of *G. mellonela*, which is not a soil insect and does not feed in the soil, was much lower with the cellular suspension providing 33% control and the filtrate providing 25% control.

Bark-Based

The next largest grouping of studies was of those which used bark-based media. Also, included in this category are bark media containing peat and coir. Efficacy across all categories, relative to internal controls, ranged from 0 to 100%. Target pest species included *Frankliniella occidentalis*, *O. sulcatus*, and *B. coprophila*. Ansari *et al.* (2008) tested several strains of entomopathogenic fungi against soil-dwelling stages of the western flower thrips *F. occidentalis.* Control levels varied among pathogen species and strains, ranging from a low of 45% with *M. flavoviridae* to a high of 96% with *M. anisopliae.* The authors also assessed whether application method, aqueous drench or homogeneous incorporation, would influence the efficacy of *M. anisopliae* applied within the same system. There were no significant differences in efficacy between the two application methods.

Along with several other soilless media types, Bruck (2006) also examined the influence of two bark based media (fir and hemlock based) on the ability of *M. anisopliae* to control black vine weevil. Conidia were pre-mixed into the soilless media. The level of efficacy was high (76–95% in two trials), and there were no differences between the two bark-based media. In another study of black vine weevil control in bark-based media using *M. anisopliae*, Shah *et al.*, (2007) obtained 100% control with an aqueous drench application, but only 65% control when the conidia were homogenously incorporated as a pre-mix. Finally, Bruck and Donahue (2007) documented 30 and 33% control of black vine weevil larvae 77 weeks after treatment with two concentrations of *M. anisopliae* pre-mixed into composted bark.

When applying aqueous suspensions of *Bacillus thuringiensis* subsp. *israelensis* to control *B.. coprophila*, Cloyd and Dickinson (2006) did not detect significant reductions in the emergence of fungus gnat adults.

Perlite

Bruck (2006) examined the ability of *M. anisopliae*, incorporated homogeneously into perlite at a concentration of 0.5lbs/y^3, to control *O. sulcatus* larvae. In two screenhouse experiments in which control was evaluated repeatedly by baiting for 133 days after treatment, he obtained an average of 81 and 91% efficacy against 6th instar larvae.

ENTOMOPATHOGENIC NEMATODES

Entomopathogenic nematodes ('EPNs') in the families Steinernematidae and Heterorhabditidae are biological control agents that are primarily used to control the soil-dwelling larval or pupal stages of insect pests. They are commonly used in highly managed systems, including greenhouse production, specialty crops, and turfgrass. EPNs occur naturally in soil world-wide (Kerry and Hominick, 2002) but are not present in composite media such as soilless mixes, except as contaminants. EPNs have a single infective stage, the non-feeding third stage infective juvenile (IJ) which is enclosed in a protective cuticle. An IJ is usually the basis of any EPN product and after application will either locate and infect a host or perish in this stage. Infective juveniles forage using a range of tactics ranging from ambushing to cruising. Ambush foragers wait for their host by elevating 95% of their body from the substrate to attach to mobile prey whereas cruisers move through the medium to locate sedentary prey. Nematodes move through the soil matrix via the water film (Wallace, 1958), which explains the importance of the water holding capacity of the substrate and the particle size (Koppenhöfer and Fuzy, 2006). Once the IJs enter their host, either through natural orifices or by penetration of the host cuticle, they regurgitate species-specific symbiotic bacteria. The nematode and the bacteria act in concert to overcome the host's immune system and host mortality follows in about 48 hours (Kaya and Gaugler, 1993). Up to three generations are produced within the host until resources are depleted and new IJs emerge from the host (Kaya and Gaugler, 1993). IJs then disperse from the host and search for a new host.

EPNs can be applied as a biopesticide in various formulations. The most commonly used method is to apply IJs in an aqueous solution (i.e., soil drench). A newer development is to apply infected host cadavers to the soil, which we will henceforth call the "cadaver treatment". With the cadaver treatment, emergence from the host is gradual, permitting a mixed-age population structure and almost continuous supply of fresh IJs. There is some evidence suggesting that IJs emerging from cadavers directly into the soil are better foragers (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Shapiro-Ilan *et al.*, 2003). The efficacy problems encountered with EPN usage could be due to a few factors, including matching proper species to the target pest and susceptible stage, but also it could be due in a large part to abiotic environmental conditions to which they are applied. One advantage of soilless media is that its components can be manipulated to reflect the ideal growing conditions for the plant, and we argue that the plants' requirements should be balanced with those of biological control agents. In one laboratory based assay against *Galleria mellonella*, the efficacy of *S. carpocapsae* was not affected by either fresh or composted bark or Sphagnum peat media (Oetting and Latimer, 1991). However, *G. mellonella* is much easier for EPNs to infect than most soil-dwelling insect larvae and differences may be apparent for other species.

The available literature comparing EPN efficacy among soilless media types is limited. The goal of most of the 12 studies reviewed here is to look at efficacy or various EPN species or application rates/ methods under greenhouse conditions. These use soilless media in the study arena, but the components of the soilless media were not always taken into consideration. By reviewing these articles, we hope to provide insight as to characteristics of soilless media that improve efficacy, or to make suggestions based on entomopathogenic nematode species.

Peat or Coir-Based Mixes

Peat-based or peat-lite soilless media are commonly used in greenhouse production and most published studies are conducted using these mixes, or commercially available mixes containing peat or coir. The use of EPNs in peat-based media and peat:sand mixes are effective against Coleopteran pests like *O. sulcatus* and *Diaprepes abbreviatus*. Nearly 100% mortality for *O. sulcatus* were obtained 14 DAT with *H. marelatus*, *H. bacteriophora*, and *S. riobrave* in a 2:1 peat:turkey grit (ground granite or clam shells) mix in greenhouse trials (Bruck *et al.*, 2005). A difference in application method was apparent with the aqueous drench solution of *H. marelatus* and *H. bacteriophora*, showing higher efficacy than the cadaver treatment. Both *Heterorhabditis* sp. were significantly different than *S. riobrave*, regardless of application method (Bruck *et al.*, 2005).

Western flower thrips stages were infected at rates up to 54% by *Thripinema nicklewoodi* (Tylenchida: Allantonematidae) in commercial peat-lite mix, Sunshine #1 (Sun Gro Horticulture, Bellevue, WA) (Mason and Heinz, 2002). A medium containing humus:clay:peat (15:35:50) that was infested with Western flower thrips (WFT) had larval mortality higher than 50% when *H. bacteriophora*, *S. feltiae*, and *S. carpocapsae* were applied (Ebssa *et al.*, 2001). Against WFT prepupae, *H. bacteriophora* and *S. feltiae* caused the highest mortality. WFT pupates in the soil, however mortality caused by EPN species was not as high as for the other life stages. *S. feltiae* had the highest mortality at 54.5%, however the results were dependant on the strain used (Ebssa *et al.*, 2001). Commercial mixes containing peat are common in studies evaluating *Scatella* sp. or *Bradysia* sp. A study by Vanninen and Koskula (2003) looked at populations of *S. tenuicosta* (shore fly) in pure Sphagnum peat moss. Preventative and repeated treatments with *S. carpocapsae* (up to 20 million/m^2) caused at least 90% mortality to *S. tenuicosta*. Repeated applications of either *S. carpocapsae* or *S. feltiae* are needed for control of *Bradysia* sp. and *Scatella* sp. Over a three week period, repeated applications of both nematode species controlled *Scatella tenuicosta* at 61–96% (Vanninen and Koskula, 2003). Two commercial mixes, Metro-Mix 366 (The Scotts, Marysville, OH) containing coir, bark, vermiculite and perlite, and Promix BX (3:1 peat:perlite plus vermiculite, Premier Horticulture, Red Hill, PA) were the media in which *S. feltiae* significantly reduced populations of *Bradysia* after 7 days compared to the control and the hardwood nursery bark mix (see below) (Jagdale *et al.*, 2004). In Pro-Mix, a single application of *H. indica* significantly reduced fungus gnat populations relative to the control from 14 to 63 DAT, while *H. marelatus* and *H. zealandica* provided control for only up to 3 and 7 DAT (Jagdale *et al.*, 2007). In a mix containing both peat and coir, *S. feltiae* provided low levels of control against *B. ocellaris* (Cloyd and Zaborski, 2004).

Peat and Sand Mixes

Peat (or peat-lite) mixed with sand has all the benefits of peat but with added weight and porosity of sand. Scarab larvae survivorship was significantly reduced by applications of *H. marelatus* in a peat:sand mix. At 21 DAT, the EPN treatments caused higher grub mortality compared to applications of the insecticide halofenozide (Mannion *et al.*, 2000). Greenhouse tests using *H. heliothidis* against *O. sulcatus* had up to 90% efficacy in a 1:1 peat:sand mix (Stimmann *et al.*, 1985). In the sandy soils typical of Florida citrus production, an EPN species, *Steinernema diaprepesi*, has been isolated that is host-specific and very long-lived (El-Borai *et al.*, 2007). A greenhouse experiment with potted citrus in peat:sand mix (1:1) examined efficacy of *S. diaprepesi*¸*H. zealanica*, and *S. riobravae* against *D. abbreviatus* 59 weeks after application with EPN species. Only *S. diaprepesi* caused significant mortality at the time of inspection (El-Borai *et al.*, 2007). *H. zealandica* and *S. riobrave* were not effective at causing weevil mortality in this experiment, possibly due to the long life cycle of the host. Both *O. sulcatus* and *D. abbreviatus* are regulated or quarantined nursery pests in the USA, and 100% control must be achieved prior to distribution of plant material. Below-ground greenhouse pests are not limited to arthropods. Plant-parasitic nematodes, especially *Meloidogyne* spp. can be serious pests, damaging the root system and overall health of the plant. EPN species *S. feltiae* and *S. riobrave* successfully suppressed *M. incognita* adults and eggs in trials using a peat and vermiculite mix (3:1, Pro-Mix) mixed equally with sand (Perez and Lewis, 2002).

Bark-Based Mixes

Most studies that look at the use of bark-based soilless mixes are aimed at controlling fungus gnats (*Bradysia* sp.). Fungus gnats are difficult to control in greenhouse production because their small body size does not allow multiple generations of EPN species within the host and little recycling of the population occurs most studies show efficacy around 50%. *Steinernema feltiae* is naturally associated with Diptera and is the most commonly applied EPN for control of *Bradysia* spp. and *Scatella* spp. (Lewis *et al.*, 2006). However, *S. feltiae* which is a cold-adapted species and does not perform well under hot conditions. In a mix of bark:peat (Sunshine SB3000 Universal Mix, Sungro Horticulture Inc., Bellevue, WA) *S. feltiae* provided around 60% control of *Bradysia* compared to the control (Cloyd and Zaborski, 2004). Harris *et al.* (1995) did not achieve significant mortality (compared to control) against *B. coprophila* 7 days after applying *S. feltiae* and *S. carpocapsae* in a bark:coir:vermiculite:perlite (2:3:2:1) mix (Metro Mix 360, Grace Sierra, Horticultural Products, Milpitas, CA). In a similar soilless mix which also contained 0–5% ash (Metro Mix 366, The Scotts, Marysville, OH) *S. feltiae* significantly reduced *Bradysia* populations compared to the control after 7 days (Jagdale *et al.*, 2004). *S. feltiae* did not control *Bradysia* sp. as well in a hardwood nursery bark mix (4:1 bark:peat), however a single application of *H. bacteriophora* significantly controlled populations up to 63 DAT (Jagdale *et al.*, 2007). Other EPN species have shown promise and deserve more study against fungus gnats. Commercially available strains of *H. bacteriophora* and *S. carpocapsae* provided reasonable control (greater than 40%) in these mixes. EPN species perform well in bark based mixes against *Bradysia* sp. and *Heterohabditis* spp. look promising when used with these mixes or with coir based media. It is possible that the high organic matter component of composted bark based mixes and the large air space enhances persistence of these nematodes which is especially important for controlling fungus gnats and shore flies. Ishibashi and Kondo (1986) had 100% kill of *G. mellonella* in laboratory bioassays for up to 6 weeks with *S. feltiae* and *S. glaseri* in sterilized and unsterilized bark media.

Other Mixes

There were no studies found during the literature search that utilized sawdust based or loam/compost based media. Saturation and poor aeration can quickly form in predominantly sawdust-based mixes due to the high water holding capacity of the sawdust. If this type of mix is used with entomopathogenic nematodes, the results will likely be similar to those of fine-textured clay soils. There were no studies to date that looked at EPNs to control greenhouse pests in a sawdust based media.

CONCLUSIONS

Drawing generalized conclusions about the effect of soilless media on entomopathogen efficacy against greenhouse pests was challenging. The effectiveness of mobile entomopathogens, like EPNs, depends on the interaction with both the soil environment and host species (Villanii and Wright, 1990). Thus, the composition of soilless media may affect EPNs to a greater extent than non-motile pathogens because the nematodes need to move through their environment to locate the host. To enhance our ability to understand the physical properties of soilless media that may influence the efficacy of entomopathogens, the characteristics of the soil should be analyzed and stated in future manuscripts. It is common practice for researchers working with mineral soils to record the pH, water potential (-kPa), percent organic matter, and soil components (particle size). If this information were available, a more thorough review would be possible. Because most of the studies reviewed lack this information, we cannot determine if these factors, such as water potential, play an important factor in efficacy. However, since most soilless media have a pH that is artificially altered, we do not suspect that pH will have much influence. We suspect that if the information were available, an increasing amount of organic matter in the substrate would positively impact entomopathogenic nematodes, fungi and bacteria. A upper threshold to organic matter must exist, but without this information, we cannot speculate on what it would be. Of all soil characteristics, for EPN species, water potential probably has the highest influence on efficacy, because it is correlated with the water film through which EPNs move.

The studies we reviewed concentrated on control of Coleopteran larvae, primarily *O. sulcatus* and *D. abbreviatus*, and Dipteran larvae, primarily *Bradysia* sp. The results vary. Pathogens applied to media containing high proportions of peat generally had caused mortality rates against Coleopteran pests than the other media types when EPNs were used. The other biopesticides did not show such a distinct pattern, but control was high in peat, coir, or peat/loam media. Combinational treatments that used either *M. anisopliae* + Neem or two EPN species against *O. sulcatus* had improved efficacy than either species alone (El-Borai *et al.*, 2007; Shah *et al.*, 2008). Entomopathogens were not as successful at reducing populations of *Bradysia* or *Scatella*, regardless of media. One study (Stanghellini and El-Hamalawi, 2005) controlled 99% of the population in a peat media with *B. bassiana*, but the majority of fungi and bacteria applications had significantly lower efficacy. Mortality against fungus gnats using EPNs has had limited success, often with a mortality rate of around 50% considered successful. The small size of the larvae limits reproduction and recycling of the nematodes. However, studies that incorporated bark based media had improved success compared with other media (Harris *et al.*, 1995; Ebssa *et al.*, 2004; Jagdale *et al.*, 2004; Jagdale *et al.*, 2007). Repeated applications of EPNs are often required to control *Bradysia* populations in greenhouses and it is possible that a preventative treatment of premixing fungi plus an EPN cadaver treatment has potential for success.

Application method is an important aspect of greenhouse management. Entomopathogens can either be applied through a soil drench (most common), premixing (for fungi and bacteria), or as cadaver formulation (for EPNs). The choice is dependant on goal of the end-user. If preventative control is desired, pre-mixing non-motile pathogens has shown to be successful. Homogenously incorporated conidia are able to persist and provide protection more than a year after application. However, curative control practices require specific action, such as aqueous drench or cadaver formulation. Both of these application methods permit choosing species-specific and strain-specific entomopathogens against the target pest. For non-motile agents, aqueous application may have the added benefit of creating a concentrated zone of pathogens through which neonate herbivore must pass, enhancing the risk of infection (Vanninen, 1999; Cloyd and Dickinson, 2006). For nematodes, cadaver formulation has shown to be superior to aqueous treatments for Coleopteran larvae (Bruck *et al.*, 2005), however, if immediate control is necessary, it may not be the preferred method due to delayed emergence of IJs. Differences between pathogen species and strain exist as well and need to be taken into consideration when applying treatments.

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