The University of South Bohemia in České Budějovice
Faculty of Science

Dissemination of *Cordyceps fumosorosea* by entomopathogenic Nematodes

Bachelor Thesis

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Annotation

C. fumosorosea is an entomopathogenic fungus that has been used as a biocontrol agent against various insect hosts for some time. Its efficacy as an entomopathogen can be increased by joined application of the fungus with entomopathogenic nematodes. The aims of this thesis are to investigate the impact of the foraging strategy of different nematode species on the spore dispersal of *C. fumosorosea* and the significance the nematode's 2nd stage cuticle holds for spore dissemination. Additionally, novel approaches for the enhancement of spore transmission in soil environments via adhesives are explored.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

Linz, Austria, 16/08/2022 ....

Miriam Hopfgartner

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Abstract

Entomopathogenic nematodes and entomopathogenic fungi are soil organisms that cause epizootics in insect pests and are therefore important agents in pest management and biocontrol. Many studies focus on the effectiveness of either entomopathogenic nematodes or fungi for controlling pests, though it has been shown that a combination of the two entomopathogens enhances their efficacy against targeted host insects. We focused on evaluating the effects of foraging behaviour of *Steinernema feltiae*, *Heterorhabditis bacteriophora*, and *Steinernema carpocapsae* on the dissemination of *Cordyceps fumosorosea* spores. Additionally, we investigated how the nematode's 2nd stage cuticle as well as various adhesives impact spore transmission. Fungal dispersal via nematodes was studied through two different experimental designs: soil-filled glass tubes and soil columns. The soil column experiment also used liquid alginate and sunflower seed oil as adhesives to explore ways to enhance spore dispersal. Results illustrated that entomopathogenic nematodes improve the transmission of *C. fumosorosea* spores; particularly conidia show a higher distribution. The nematode strains *S. feltiae* and *S. carpocapsae* were found to spread spores most effectively, depending however on the experimental soil conditions. Research revealed that the nematode's 2nd stage cuticle is an important factor for spore dispersal; without it, transmission rates drop significantly. Sunflower seed oil was determined to be the most successful adhesive medium and increased spore distribution for all tested nematode strains. These findings indicate the potential use of adhesives in pest management to increase efficacy. Additional research regarding adhesives, as well as the mechanisms of spore adhesion to the nematode cuticle may be of importance in the future.

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1. Introduction

1.1 *Entomopathogenic Nematodes*

Nematodes, belonging to the phylum Nematoda, are unsegmented roundworms that constitute one of the most abundant groups of organisms on earth. Taxonomically, they belong to the super-phylum Ecdysozoa, which encompasses organisms that possess and shed cuticles. [1] It is estimated that around 500.000 to 100.000.000 species, most of them currently undescribed, exist. [2]

The nematode families Steinernematidae and Heterorhabditidae (order Rhabditida) occupy a unique position since species of these families found a use as biocontrol agents. Qualities that set these families apart from others include:

- the opportunity for large-scale culturing,
- their ability to transfer their symbiotic bacteria into the body cavity of insects, and
- their wide range of possible hosts. [3]

Characteristic for entomopathogenic nematodes (EPNs) is their symbiotic relationship with bacteria. *Steinernema* spp. form a symbiosis with *Xenorhabdus* spp., whereas in *Heterorhabditis* spp. the symbiotic bacteria are *Photorhabdus* spp. [4]

1.1.1 *General Morphology*

Nematodes possess a body wall made up of a cuticle and a singular, longitudinal muscle cell layer, with a thin hypodermis separating the two. [5] A thick cuticle covers the unsegmented body and lines the buccal cavity, oesophagus, excretion pore, anus, vagina, and cloaca. The cuticle itself is covered by an epicuticle with a surface rich in carbohydrates. Formation of the cuticle takes place in three areas on the body: the cortical, median, and basal regions. [6] Additionally, advanced age of the organism correlates to increased thickness of the adult cuticle, which is due to growth of the basal area. [7] As opposed to the multi-layered body cuticle, the cuticle covering the orifices seems to be single-layered and secreted by underlying cells. [8] Generally, the cuticle can appear smooth or be adorned with rings, alae, spikes, or longitudinal ridges.

Characteristic roundworm-nervous-systems consists of ventral and dorsal nerve cords that surround the pharynx and run towards each end. The central nervous system includes the ventral nerve bundle, interneurons, and ganglia. [9]

A notable chemo-sensory organ that occurs in all nematode species is the amphid which is positioned bilaterally in the head region. This sensory organ is of importance for species

identification since its shape can vary, with a general distinction between spiral and non-spiral amphids. [8]

1.1.2 Morphology of Heterorhabditidae

Nematodes belonging to this family maintain morphological characteristics that resemble those of free-living Rhabditidae-species. Especially in males, which can only be found in the second generation inside insect cadavers, these qualities are present and include transparent, rod-like bodies. This body form is in direct opposition to the female's which has a more sausage-like appearance.

Distinct features of Heterorhabditidae-males include a tube-shaped buccal cavity, a club-like pharynx, as well as a bursa copulatrix, also typical features of Rhabditidae.

In females of Heterorhabditidae, a dark intestine is visible when illuminated with a light microscope. Their auxiliary excretory system is a remnant of the Rhabditidae ancestry. [4] [6] Additionally, juveniles can be seen moving around inside of older female's bodies, where they go through approximately two moults.

The third stage IJs of Heterorhabditidae are enveloped by their second stage cuticle, thereby becoming "dauer" juveniles. The surface of the third stage cuticle is smooth and the IJs possess a tooth on the anterior end.

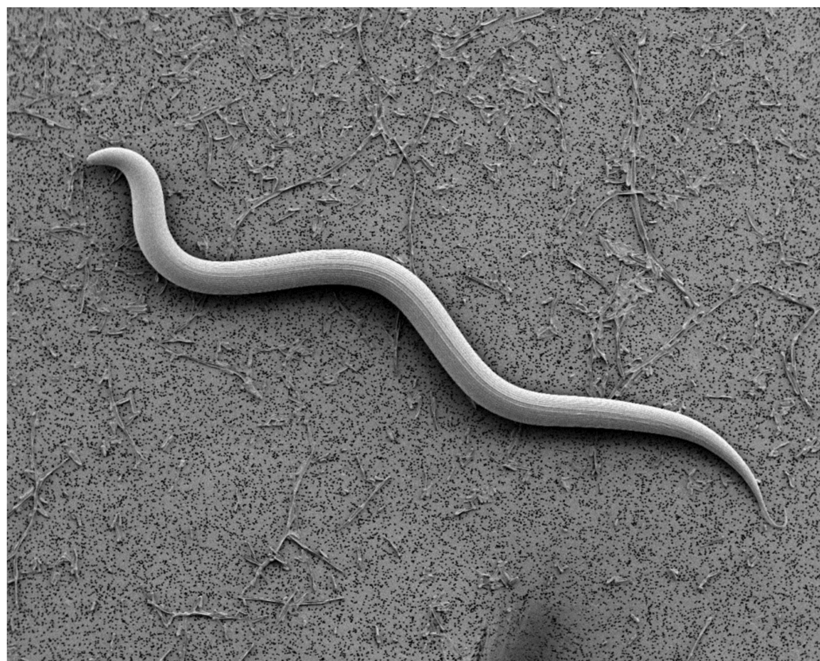

Figure 1: Image of a *H. bacteriophora* individual surrounded by blastospores of *C. fumosorosea*

1.1.3 Morphology of Steinernematidae

A key difference in morphology between Steinernematidae and Heterorhabditidae is the absence of the caudal alae in the tail region of Steinernematidae-males. One prominent feature of males belonging to Steinernematidae is a median papilla located in vicinity to the cloaca. Similarly to Heterorhabditidae, the body shape of Steinernematid-females is sausage-like with gonadal tubes and intestines taking up space inside the body.

Within the family of Steinernematidae, two distinct forms of spermatozoa can be observed. Species like *S. feltiae* possess chains of amoeboid spermatozoa, whereas massive amoeboid megaspermatozoon with surface-attached microspermatozoa are found in species like *S. carpocapsae*.

IJs of this family shed their second stage cuticles soon after departing their host's cadaver. Their third stage cuticle shows longitudinal ridges on both sides of the body, which exhibit a great deal of diversity within the genus. [4]

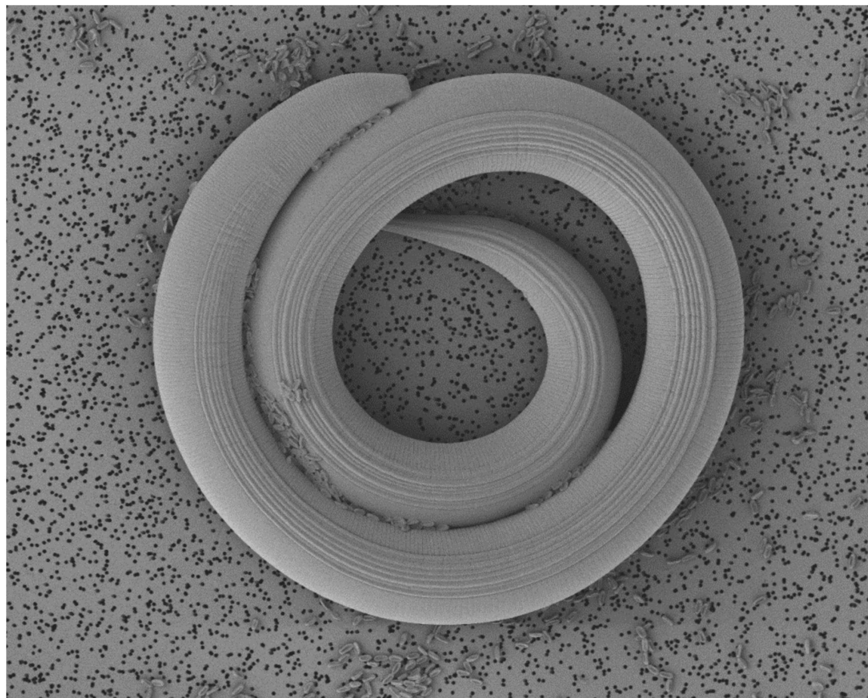

Figure 2: Image of a *S. feltiae* individual surrounded by conidia of *C. fumosorosea*

1.1.4 Symbiosis of Nematodes and Bacteria

Both the nematode families *Steinernema* and *Heterorhabditis* maintain symbiotic relationships with gram-negative bacteria. Namely, *Xenorhabdus* spp. live in symbiosis with *Steinernema* and *Photorhabdus* spp. with *Heterorhabditis*. *Steinernema* store their symbiotic bacteria in a designated part of the intestine, the so-called "intestinal vesicle" [10]. In contrast, *Heterorhabditis* symbionts are present in the anterior part of the intestine. [11]

The symbiotic bacteria are subsequently released from the gut into the host's haemolymph after penetration through an IJ. Next, the bacteria begin to produce toxins which typically kill the host within 24 to 48 hours. Bacteria then multiply inside the host and serve as a food source for the nematodes, which allows them to moult and multiply. [12]

While the symbiotic bacteria are mainly responsible for the swift killing of the hosts, the nematodes shield their symbionts from the environment and, in some cases, impede the immune response of the host insect.

Generally, the nematode-bacteria symbiosis is non-obligate, and culturing of both species individually is possible. However, in combination they show a high degree of specificity.

The interaction of the symbiotic bacteria with the nematode takes place on two levels: a phoretic and a vegetative one. Retention of the bacteria and interaction with the intestine of IJs marks the phoretic state where multiplication appears to be subdued. On the other hand, in the vegetative state, the host's defence mechanisms are overridden by the bacteria, therefore enabling unrestricted multiplication. [13]

1.1.5 Life cycle of Entomopathogenic Nematodes

The general life cycle of nematodes usually begins with a female laying eggs. Typically, development of the juveniles takes place inside the egg before hatching, up until the second juvenile stage (J2). Through moulting and feeding the juveniles evolve to the fourth juvenile stage (J4), also known as the pre-adult stage. Nematodes have one infective juvenile stage; in EPNs this is the third juvenile (J3) stage.

Distinction of the different juvenile states is generally possible, because of the occurrence of specific structures like the reproductive or digestive systems. [14]

Inside host insects *Steinernema* experience two amphimictic generations, whereas *Heterorhabditis* undergo one asexual generation which is followed by an amphimictic one.

The process of host infection is divided into three steps: Initially a migration period occurs where the nematode searches for a host. The second step is distinguished by the penetration of the host insect. Nematodes enter the host through natural body openings, for example

mouth, anus, and spiracles. *Heterorhabditis* additionally possess a tooth, which allows them to enter the haemolymph through the insect's body wall as well. Lastly, in collaboration with its symbiotic bacteria, the nematode overcomes the host's immune response, and a new generation of juveniles is created. These juveniles will then leave the host insect after adequate multiplication and depletion of nourishment to start the cycle anew. [15]

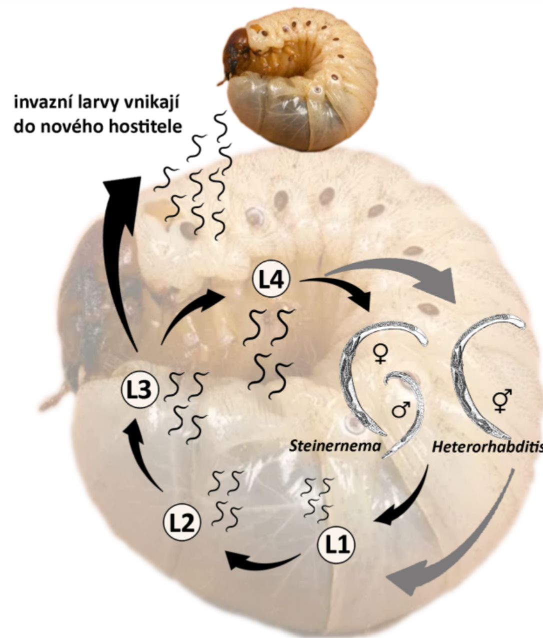

Figure 3: Illustration of the life cycle of *Steinernema* and *Heterorhabditis* EPNs

1.1.6 Foraging behaviour

Different species of nematodes employ diverse foraging strategies, which is of importance in integrated pest management (IPM). These foraging behaviours range from ambushers over intermediates to cruisers. While ambushers can be employed to control populations of mobile insects, cruisers are commonly better suited for stationary insects. [16]

Ambushers like *S. carpocapsae* are mostly found on or near the soil surface where they wait for potential hosts. Once an insect passes by, infection occurs when the ambusher IJs jump on them. Response to host volatile cues is not found in ambusher species and location of the host only takes place after direct contact was made. [17] Some *Steinernema* species can also stand on their tails, enabling them to have most of their bodies in the air. This behaviour is known as nictation. [15]

H. bacteriophora is an example of a nematode species which employs the Cruiser strategy. These nematodes are highly active and move through the soil profile, which leads to their distribution in the soil. Nictation has not been observed in Cruisers however, a response to carbon dioxide, which is released as a cue by the host, has been detected. [18]

An intermediate strategy has been adopted by species like *S. feltiae*. Characteristic for this strategy are attacks on both mobile and stationary insects. Therefore, these nematodes can be found either at the soil surface or deep within it. [4]

1.1.7 Ecology

Nematodes can persist in all habitats except for air and water, but even these unwelcoming environments can be indirectly occupied through parasitism. Organisms such as birds, arthropods, and fish present suitable hosts inhabiting these locations. [5] The soil environment is generally a more shielded one, however the IJs of EPNs might still experience unfavourable conditions. Three main factors that influence nematode survival in the soil have been identified: abiotic and biotic stresses as well as energy reserves.

In the group of abiotic stresses, desiccation and extreme temperatures were found to be the most significant threats to nematode survival. [19] Since nematodes rely on water to ensure their movement, desiccation poses a significant threat. When exposed to desiccation, at first the organism becomes less active, but with further desiccation water is lost. The tolerance of *Steinernema* and *Heterorhabditis* to desiccation is limited, and these families can therefore be considered partial anhydrobiotes. [13]

Extreme temperatures are generally damaging to nematodes; however, the extent of damage depends on the length of exposure. *Steinernema* and *Heterorhabditis* are able to withstand temperatures below 0 °C for several days. [20] High temperatures can be fatal, yet there are certain species equipped to endure them. [5] In general, temperatures of 30 °C and above impede reproduction as well as host infection, thus heat resistance is only a marginal characteristic attributed to specialised species. EPNs low toleration of desiccation, high temperatures and ultraviolet light thus indicates their evolution as soil-dwelling organisms.

In the category of biotic stresses mainly predators that affect survival of EPNs in the soil, such as collembolans, predatory mites, or parasitic fungi, are found. [13]

1.1.8 Use in Biocontrol

Various taxa of entomopathogenic nematodes have been found to exhibit potential as biological control agents. However, Heterorhabditidae and Steinernematidae were revealed to be the most successful since they possess many qualities that are necessary for usage in pest control. [13] [12] Additionally, extensive research has been done that illustrates their successes as well as shortcomings in controlling insect pests that target turf, lawn, crops, and ornamentals. [21] [22]

Generally, entomopathogenic nematodes can be seen as promising contenders for integrated pest management and sustainable agriculture. The advantages that make EPNs favourable subjects include:

- their broad host ranges,
- the ability to actively seek out and ambush hosts (dependent on the characteristics of the chosen species),
- the ability to swiftly kill the targeted pests (within 48 to 72 hours),
- the possibility of mass production *in vivo* or *in vitro*,
- usage of EPNs is generally considered safe for non-targeted invertebrates and vertebrates, and
- it is possible to apply EPNs with standard equipment.

On the other hand, disadvantages that have been identified include:

- the cost of manufacturing,
- requirement of refrigeration for long term storage and limited shelf life, and
- environmental limitations, involving moisture, temperature, radiation, soil chemistry and pesticide use. [22], [23]

The selection of a fitting EPN for a specific pest is crucial for the success of the management strategy. Factors that should always be considered when choosing an appropriate species involve the host range, which can widely differ between strains, the foraging strategy and the nematode's tolerance to environmental aspects. [24], [25]
For application in subterranean habitats (against white grubs or weevils) as well as foliar or cryptic ones, EPNs with mobile foraging behaviours like cruisers or intermediates, could be of use. This is based on the assumption that the basic requirements of a receptive host,

complimentary temperature and moisture are met. Contrastingly, ambushers, who employ a sedentary strategy, are more suited towards soil surface or cryptic habitats. [22]

An important aspect that should not be neglected is the environmental impact that could come along with the usage of biological control agents. Although evidence in this field is overall limited, available studies suggest general safety of EPNs with little impact on the environment. [26] [27] Especially in current times, where there are tendencies to decrease the use of chemical pesticides, EPNs seem to present a promising alternative. [28] When comparing EPNs with conventional chemical pest control, it is suggested that the environmental impact of EPNs may be considerably smaller, indicating their superiority. [29]

1.2 Entomopathogenic Fungi

Entomopathogenic fungi (EPFs) are an abundant group in nature that are globally distributed, except in regions of the Arctic and Antarctic. [30] After contact with a host, they cause epizootics and ultimately kill the insect. The most important order of EPFs for biocontrol is the order Hypocreales (family: Cordycipitaceae) which include notable EPF species that are already used for pest control and possess a wide host range. Examples of these species are *Beauveria bassiana*, *Metarhizium anisopliae* and *Cordyceps fumosorosea.* [31]

Nevertheless, for the formulation of biopesticides, strain selection is of importance and may be even more important than species selection. Additionally, virulence may vary depending on the insect's life stage. [32]

The EPF strain used in this study, namely *Cordyceps fumosorosea (formerly: Isaria fumosorosea)*, has gained attention over the last fifteen years, due to its use as a bioagent against white flies or thrips. [30]

1.2.1 Life cycle

EPFs can be found in nature either as spores or resting (dormant) spores, to survive times of environmental stresses. Infection of a host insect by EPFs commences when the insect encounters the asexually produced conidia, which can be found in the air, in soil, on plants or on cadavers of previous hosts. [32] The life cycle of EPFs consists of a parasitic phase (infection of the host and subsequent death) followed by a saprophytic phase (after the death of the host) [33].

The beginning of pathogenesis is marked by the adhesion of conidia to the insect's cuticle. The adhesion process is assumed to include a non-specific mechanism which is facilitated by the hydrophobicity exhibited by the cell wall of conidia. [34] After initial contact, germination, including formation of structures for penetration (e.g., germ tubes, appressoria, penetration pegs, extracellular sheath), takes place, followed by the formation of hyphae. Under suitable conditions, penetration of the host cuticle may take place through a blend of mechanical as well as enzymatic mechanisms. Once the fungus has surpassed the cuticle, invasion of the host body and circulatory system via the haemolymph ensues. Hyphal bodies (blastospores) are produced and spread throughout the body, thus leading to death of the host insect. Death typically occurs three to seven days post infection due to starvation via nutrient depletion, invasion of organs or release of toxins by the fungus. The production of metabolites (e.g., Beauvericin, Oosporein, Destruxin B., etc.) may influence the fungus' ability to prevent growth of competing microorganisms on the cadaver's surface.

Following host-death, the fungus continues its growth inside the host saprotrophically and under sympathetic conditions, hyphae arise on the outside of the cadaver. Through sporulation, a second generation of conidia is released and dispersed passively by wind, rain or contact with biological vectors. [32], [35], [36]

Figure 4: Hyphal growth of *C. fumosorosea* on the outside of a dead host insect

1.2.2 Factors influencing the effectiveness of entomopathogenic fungi

The effectiveness of entomopathogenic fungi is directly influenced by the action of abiotic (temperature, humidity, and UV radiation) and biotic factors (fungus-host interactions). [37] These environmental factors have a particular influence on the germination, vegetative growth as well as the viability of EPFs. [38]

1.2.2.1 Biotic factors

Some biotic factors are able to influence the infectivity and stability of EPFs. Particularly in *C. fumosorosea* the efficacy may be dependent on the fungal isolate or the propagule, either conidia or blastospores, used. [39] A connection between spore length, germination speed and the infectivity of assorted *C. fumosorosea* isolates on *Plutella xylostella* larvae has been experimentally discovered as well. [40] In addition to this, the fitness of the host (*P. xylostella*), i.e., whether the larvae have been fed or starved, impacts their predisposition to fungal infection through different strains. [41]

Generally, an assortment of different morphological and physiological features could influence an insect's susceptibility to EPFs. [42], [43] These factors include population density, nutrition, age, as well as injury to the insect. It has also been found that stress plays a role in host susceptibility to infection. While some features like population density and nutrition, in addition to exposure to chemical stressors and environmental factors, seem to increase stress in insects, they also predispose them to attacks by entomopathogens. However, the influence of the environment and physiological mechanisms, like low immune response, are poorly understood up until now. [35]

1.2.2.2 Abiotic factors

Important abiotic parameters include the temperature, solar radiation, availability of water and relative humidity (RH), precipitation and wind. [30], [35]

The temperature tolerance of EPFs is relatively high [35], but significantly affects conidia germination, growth, and reproduction in the host body as well as sporulation. [44] Therefore, temperature proportionally affects the length of the development cycle and is one of the critical factors in the effectiveness of entomopathogenic fungi [37]. In general, the optimum temperatures for germination and growth are in the range of 20-30 °C. [35]

Cordyceps fumosorosea is a mesophilic fungal species, whose temperature range for optimal growth therefore also lies between 20 to 30 °C. Although, variations between different strains which stem from diverse locations can be observed. [45]

Solar radiation has been shown to have detrimental effects to survival of conidia spores of EPFs, with *C. fumosorosea* being particularly susceptible to damage. It has been found that UV- B radiation is the most potent for spore destruction, followed by UV-A radiation. Infrared radiation seems to have a less harmful effect. [46], [47] UV protectants are often used in biopreparations against the negative effects of sunlight. Some carriers, such as oil- or clay-based carriers, provide protection against the harmful effects of UV radiation and can at least prolong the vitality and survival of conidia in field conditions. [48]

Relative humidity (RH) has been found to influence the efficacy of EPFs in various ways. For example, when applying EPFs for IPM, the evaporation of spray droplets can be influenced through humidity in conjunction with temperature. This may in effect cause disappearance of small droplets and therefore influence the targeting. [49]–[51] The lower limit between 90 and 95% relative air humidity is considered the critical limit for conidia germination. When the relative airiness drops below the lower limit, conidia germination decreases, and the success rate of infection is reduced. [44] Additionally, moisture can influence the perseverance of the fungal inoculum. Mostly, it was found that the conidia of EPFs are the most stable when conditions are cool and dry, however this is strongly dependent on the species and fungal strain that is employed. [49]–[51] Furthermore, rainfall can serve as a vector to disperse conidia and propagules. [35]

1.2.3 Cordyceps fumosorosea

According to the latest phylogenetic studies, the genus *Cordyceps* is classified in the order Hypocreales, family Cordycipitaceae, in contrast to the species that are still classified as genus *Paecilomyces*, belonging to the family Clavicipitaceae. [52]

1.2.3.1 Morphology

The fungus *C. fumosorosea* produces a white, cotton-like mycelium on the host, which later turns pinkish, or purplish to grey-purple. Older, fully sporulating cultures even show a grey-purple coloration and the cotton-like character of the colony changes to a dustier one. [30], [54] Within the genus, well developed, synnematous conidiophores are usually present. The conidia itself are single cells that tend to be oval-shaped [32], hydrophobic and gradually separate at the end of the phialides (conidiogenous cells of which there are 3-6 per conidiophore). The youngest conidia is always in contact with the phialide and pushes older conidia further into the forming chain. [30]

Production of the fungus can take place via two different methods, where each yield a different spore type. Solid-state fermentation produces aerial conidia, whereas submerged liquid-state fermentation yields blastospores. [53]

1.2.3.2 Host range

The *C. fumosorosea* species complex exhibits a relatively large range of hosts, where Lepidoptera dominate. However, if compared to *B. bassiana*, the range is significantly narrower. [33] A first list of hosts of *I. fumosoresea* compiled in 1970 recorded only three species of lepidopterans and one coleopteran, but later researchers found over 40 species susceptible to the fungus. [55], [56] Potential hosts are found in the orders:

- Acari
- Blattodea
- Coleoptera
- Diptera
- Hemiptera
- Hymenoptera
- Isoptera
- Lepidoptera
- Neuroptera
- Thysanoptera [30]

1.2.4 Transmission and Spore Dispersal

Transmission of conidia is primarily facilitated by wind, however rain as well as invertebrates play an additional role in spore distribution. Furthermore, hyphae which grow from insect cadavers are important for dispersal of conidia. Moreover, for germination and sporulation to take place the requirements of high humidity and soil moisture must be met. Infection of multiple life stages of an insect host may also be advantageous in spreading of the disease. [32] Generally, the capability of EPF spores to infect potential hosts through their cuticle instead of through ingestion represents a great advantage for their dispersal. [57]

The hydrophilic, vegetative blastospores are the preferred growth form of EPFs inside the hemocoel of the host. Due to their yeast-like growth form, they are better adapted to nutrient uptake from inside the host and can even be deemed virulence factors. [58] The germination rate of blastospores in *C. fumosorosea* has been found to be significantly higher than that of

conidia. Over 90% of blastospore germination takes place within six hours, in contrast to sixteen to twenty-four hours for conidia. Additionally, a faster germination rate might also be beneficial for reduction of exposure to environmental stresses like UV-radiation, temperature, and humidity. Since blastospores have a lower ability to tolerate these stresses than aerial conidia, faster germination seems to be preferred. [59] In turn this however leads to a reduced shelf life of blastospores under ambient conditions, which must be considered especially when they are not directly sprayed onto plants. Still, it should be considered that blastospore virulence is species-dependent with some, like *Metarhizium anisopliae* or *Hirsutella thompsonii*, exhibiting lower virulence and stability of blastospore than conidia. [40], [41]

1.2.5 Use of EPFs in Biocontrol

The main application approaches used for EPFs in IPM are:

- Classical biological control,
- Augmentation (inoculative, inundative),
- Conservation.

When used for epigeal environments, entomopathogenic fungi have been mostly applied using an inundative approach, whereas soil environments have been mostly subjected to inoculative or inundative application strategies. Generally, entomopathogenic hyphomycetes can be classified as being comparatively slow acting or having sublethal effects. Their efficacy in field applications also depends on environmental factors (see: Factors influencing the effectiveness of entomopathogenic fungi). If conditions are ideal, after application of EPFs the time until death of the target pest lies between three and five days, however in field experiments longer time frames must be expected. Sublethal effects of EPF infection are a somewhat neglected area. The term describes infections not leading to death which however still impede the insect's biology. [35] An example is the reduction of reproductive potential in Colorado potato beetles after application of *B. bassiana*. [62]

For example, *C. fumosorosea*, and in particular its Apopka strain, have received considerable attention and products for pest management based on this fungus or in combination with other species are commercially available. [30] A significant amount of research into the use of *C. fumosorosea* against whiteflies, especially *Trialeurodes vaporariorum, Bemisia argentifolii,* and *Bemisia tabaci*, has been done, establishing the fungus as one of the most important entomopathogens for control of these pests. Numerous virulent strains against

whiteflies have been found in the *C. fumosorosea* species complex. Research shows that under optimal conditions infection and death of whiteflies is swift, where death occurs in 24 to 48 hours and conidiogenesis arises within 72 hours on the cadaver's surface.[63] Inundative field application of *C. fumosorosea* conidia or hyphal bodies on field crops yielded > 90% mortality, especially in nymphs. [64]

Nevertheless, limitations of EPFs may involve:

- slow action in comparison with alternative insecticides,
- somewhat high costs,
- limited shelf-life,
- dependency on environmental conditions,
- decreased efficacy upon synchronous application of fungicides.

To lessen or compensate for the limitations of EPF use, suggestions include:

- focus on early developmental stages of pests in treatment,
- non-simultaneous application of EPFs and fungicides,
- focus on insect populations in moderate environments,
- application on crops susceptible to multiple spray applications [30].

On the other hand, advantages for EPF use in biocontrol include:

- EPFs do not pose as a threat to non-targeted, beneficial organisms and thereby maintain biodiversity [65],
- Their ability for mass production of inoculum [36],
- Potential to replace chemical pesticides in conventional food production, or use in organic farming where they are already prohibited [66],
- Harmlessness towards humans and vertebrates [33].

Additionally, a significant advantage of *C. fumosorosea* strains for IPM is the fungus' general compatibility with non-target or beneficial organisms. Research in this area was mostly conducted regarding the usage of the fungal species in biocontrol. [30]

In experiments with thrips (*Ceratothripoides calaratis*), five *C. fumosorosea* strains were deemed highly effective, yielding mortality rates between 80 and 93%. [67]
Another study found that in a field experiment against onion thrips (*Thrips tabaci*), a combined application of *C. fumosorosea* and the nematode species *S. feltiae* showed a significant decline in insect numbers per plant. The frequency of infestation dropped from 93% in the control to only 38%, [68] therefore illustrating the advantages of using a combination of entomopathogens (EPNs + EPFs), as is done in this present study.

2. Aims of the Thesis

- Evaluation of the effect of foraging strategy of entomopathogenic nematodes *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on dissemination of conidia and blastospores of *Cordyceps fumosorosea*.
- Assessment of the effect of nematodes' 2nd stage cuticle on the spores of *C. fumosorosea* dissemination.
- Suggest a method for enhancement of spore dissemination in soil environment by using adhesives.

3. Materials and Methods

3.1 Model Organisms

To conduct the experiment, the nematode strains *Heterorhabditis bacteriophora* (HB221), *Steinernema feltiae* (NFUST) and *Steinernema carpocapsae* (1343) were used.
All three strains are sustained in the nematode collection that is housed in the Laboratory of Entomopathogenic Nematodes, at the Institute of Entomology, which is part of the Biology Centre of the Czech Academy of Science (Czech Republic).
The strain CCM8367 of the fungus *Cordyceps fumosorosea* which originates from the horse chestnut, *Cameraria ohridella*, collected in the Czech Republic, was used for the experiments. The strain is patented and deposited in the Czech Collection of Microorganisms in Brno. The fungus is grown on Petri dishes (∅ 90 mm) that are incubated at 25 °C in a dark fridge for 14 days. A selective medium of potato dextrose agar (PDA, Sigma-Aldrich, Munich, Germany), to which dodine was added to ensure growth of the target fungus species only, was used.

3.1.1 Preparation of Nematodes

Culturing of the nematodes took place *in vivo*, inside *Galleria mellonella* larvae. A supply of the larvae is permanently maintained in the Laboratory of Entomopathogenic Nematodes. To limit silk production, the larvae are subjected to a temperature of 58 °C for 10 second and are afterwards stored in a dark fridge. Survival is possible for up to two months at temperatures of 10 °C.
The larvae were placed in an infection chamber, which consisted of a 90 mm Petri dish with slightly wet filter paper on the bottom, and infected with approximately 50 IJs per larvae. These chambers were stored in the dark, at room temperature, for three to five days.[14]
The next step was collection of the dead larvae by placing them on White traps to collect the IJs. The closed White trap with sterile water in the outer Petri dish was left to stand at room temperature until emergence of IJs. The IJs were then acquired from the white trap and subsequently stored at 5 °C in sterile tap water in the fridge. [14]

3.1.2 Preparation of Spores

Because the experiment demanded the use of conidia as well as blastospores, two different harvesting methods had to be employed; both performed in a Flowbox under sterile conditions. The hydrophobic conidia were harvested by pouring 0.05% (v/v) sterile Tween solution onto the Petri dish and using an inoculation loop to detach the spores. The

suspension was then poured into a glass tube that was vortexed for a few seconds. The homogenised mixture was consequently filtered over sterile gauze to separate the spores in solution from the mycelium. The conidia-solution was then diluted 100 times to make spore counting possible. For counting of the spores, a Neubauer improved counting chamber was used. The concentration of conidia was adjusted to 1 x 10^7 spores per mL.

The hydrophilic blastospores were cultivated in potato dextrose broth (PDB). Into a 250 mL Erlenmeyer flask containing 95 mL of PDA, three pieces of mycelium were placed. The mycelium was detached using an inoculation loop. The solution was then positioned on an orbital shaker to incubate (200 rpm, 25 °C) for four days. Afterwards, spores were collected by filtering the solution over sterile gauze to separate them from the mycelium. The spore-concentration was determined with a Neubauer improved counting chamber and adjusted to 1 x 10^7 spores per mL.

3.2 Glass tube experiment

The experimental apparatus consisted of a 10 cm long glass tube, with parchment paper on the inside. Slightly wet, sterilised brown soil was filled into the tubes and two 2 mL Eppendorf tubes were attached to each end of the glass tube. To fasten the Eppendorfs, large perforations were made in the lids. Additionally, small pieces of synthetic fabric (Uhelon 130T) were stuck to the bottom part of the Eppendorf lids, to shield the tubes from the soil. A *G. mellonella* larvae was placed in one of the Eppendorfs to be used as bait and provide an incentive for the nematodes to travel through the soil-filled glass tube. The other Eppendorf tube was left empty. However, on this side 20 µL of *C. fumosorosea* spore suspension as well as a suspension of nematodes in sterile water was applied. The amount of nematode suspension was adjusted to give a total of 500 IJs per glass tube. [69]

Altogether, 10 of these glass tube apparatuses containing spores and nematodes (F + N), plus five controls only including fungal spores (F), were prepared for each experiment run. An experimental series was performed with this setup, using the nematode species *S. feltiae*, *H. bacteriophora* and *S.carpocapsae* in combination with conidia and blastospores of *C. fumosorosea*.

For the sake of investigating the significance of the IJs second stage cuticle in spore transmission, additional runs of the experiment were carried out. A 1% sodium hypochlorite (NaOCl) solution was employed to de-sheath the IJs. Five minutes of exposure were

sufficient for de-sheathment. Data was collected with all three model species and both conidia and blastospore dissemination was measured.

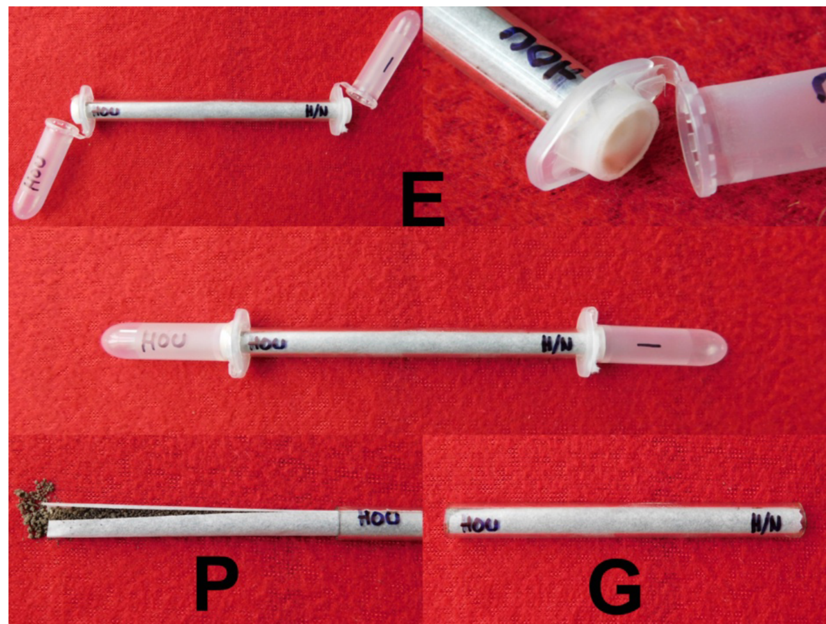

Figure 5: Experimental design of the glass tube experiment.
Eppendorf tubes (E), parchment paper (P), glass tube (G)

3.2.1 Measuring of spore concentration in glass tube experiment

The spore concentration in the soil was measured three days after application. To facilitate this, each glass tube was divided into three parts. The soil was suspended in sterile Tween solution, where the amount used was varied depending on the part. The end of the glass tube where fungal suspension was applied, was designated as part one and 100 mL of Tween solution in a 250 mL Erlenmeyer flask were used for suspending. For parts two and three, 5 mL and 3 mL respectively were adequate. Homogenisation of the samples was accomplished on an Orbital shaker for part one or with a Vortex for parts two and three. The Orbital shaker was operated at 25 °C for 20 minutes at 200 rpm.

Afterwards, part one samples were diluted to a ratio of 1:100 with Tween solution, while parts two and three were left undiluted. Of each sample, 0.5 mL were transferred onto Petri dishes with the selective medium (PDA + dodine) and spread evenly with L-shaped wipers. Two Petri dishes for each sample were prepared this way. In total, 90 Petri dishes per experimental run were obtained.

After a week of incubation at 25°C, the number of *C. fumosorosea* colonies on each Petri dish was counted. Results are expressed in colony forming units (CFU) per mL of soil.

3.3 Soil column experiment

This experiment was performed on 9 cm plastic Petri dishes with *C. fumosorosea* selective agar medium (distilled water 900 mL, PDA 36 g, cycloheximide 0.225 g, chloramphenicol 0.45 and dodine 0.045 g).

Soil columns (see Figure 6) were constructed from 15 ml plastic falcon tubes cut to a length of 3 cm. Textile mesh was glued onto the bottom of the tube and the obtained column was filled with sterilized, slightly wet, brown soil.

Next, nematodes (1 000 IJs in 40 μl of water), fungus (40 μl of adjusted suspension) or both nematodes and the fungus were added into 120 μl of sterile tap water, 1% alginate gel or sunflower oil respectively and total volume of 200 μl was transferred onto the top of the soil columns.

The prepared columns were then placed on the Petri dishes, each with one *G. mellonella* larvae immobilized in an iron cage to attract nematodes and support their movement. After preparation of the dishes as described, they were placed in plastic boxes (15 x 10 x 8 cm) to be stored at room temperature (21°C) for 5 days.

Subsequently, the *C. fumosorosea* colonies were counted and recorded as the total number CFUs. For the experiment, 10 dishes were used for each combination: fungus only (F), nematodes only (N), fungus/nematodes (F/N) applied in tap water, sunflower oil, or liquid alginate in combination with conidia or blastospores and ex-sheathed or en-sheathed IJs of all three EPNs´ species already mentioned above. This gives 60 combinations altogether, which equals 600 Petri dishes used over the entire trail. The whole experiment was repeated twice.

Figure 6: Experimental design of the soil column experiment. Pictured here: soil column in plastic box, column on its own, soil filled column on Petri dish

3.4 Statistical evaluation

The obtained data was evaluated using General Linear Model (GLM) and all variables were normalised using logarithmic transformation. For drawing of the column graphs raw (non-transformed) data was used. The Tukey post-hoc test was employed to test for differences among variables. Analysis was wholly performed in the program Statistica 10 (StatSoft Inc.).

4. Results

4.1 Glass tube experiment

When analysing the collected, it was found through statistical testing (see Table 1) that the number of transmitted spores by nematodes significantly ($p<0.001$) depends on the position in the glass tube, the presence of the nematode's 2nd stage cuticle, the nematode species used, presence of nematodes in general, as well as the spore type.

Table 1: Complete results of statistical analysis (GLM) for the Glass Tube Experiment. Red colour indicates statistically significant result ($p < 0.05$).

	d. f.	F	p
(1) Position in glass tube	2	2016.255	<0.001
(2) Presence of 2nd stage cuticle	1	15.114	<0.001
(3) Nematode species	2	9.366	<0.001
(4) Presence of nematodes	1	36.454	<0.001
(5) Spore type	1	13.970	<0.001
1*2	2	2.314	0.100
1*3	4	9.811	<0.001
2*3	2	20.184	<0.001
1*4	2	0.908	0.404
2*4	1	30.719	<0.001
3*4	2	4.557	0.011
1*5	2	0.441	0.644
2*5	1	3.037	0.082
3*5	2	1.117	0.328
4*5	1	1.583	0.209
1*2*3	4	12.328	<0.001
1*2*4	2	4.766	0.009
1*3*4	4	3.541	0.007
2*3*4	2	4.520	0.011
1*2*5	2	0.122	0.885
1*3*5	4	0.414	0.799
2*3*5	2	0.811	0.445
1*4*5	2	1.704	0.183
2*4*5	1	0.000	1.000
3*4*5	2	0.150	0.861
1*2*3*4	4	4.642	0.001
1*2*3*5	4	0.559	0.693
1*2*4*5	2	1.547	0.214
1*3*4*5	4	1.227	0.298
2*3*4*5	2	0.816	0.443
1*2*3*4*5	4	1.119	0.347
Error	468		

Further statistical analysis shows that the overall dissemination of spores, regardless of type, was significantly increased when both nematodes and fungal spores were applied to the glass tubes, in comparison to simple spore application. Especially the *S. feltiae* strain NFUST was found to considerably increase ($p<0.05$) spore transmission. Nevertheless, an increase in transmission can also be seen with the strains *H. bacteriophora* HB221 and *S. carpocapsae* 1343 when compared to the control. Additionally, *H. bacteriophora* was shown to transmit a higher number of spores further through the glass tube (towards the end with the bait). In contrast, *S. feltiae* showed a drop in transmission between the second and third part of the tube, while *S. carpocapsae* exhibited lower rates of transmission in general.

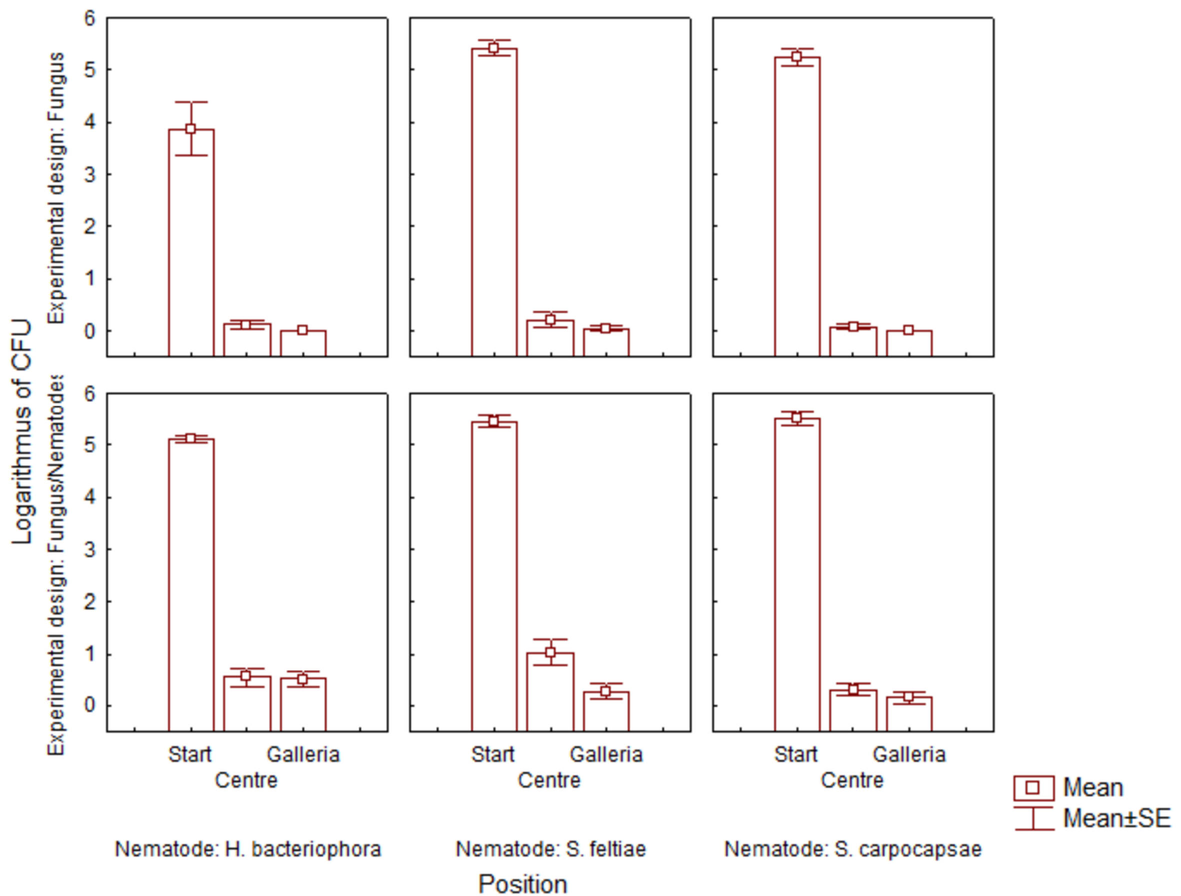

Figure 7: Logarithmically transformed number of Colony forming units (CFU) with mean (± SE) for the control (fungus application only) and the experimental runs (application of one out of three nematode species and fungus). Number of CFUs per part of the glass tube is given in individual columns (Start, Centre, *G. mellonella* larvae)

When comparing the transmission of conidia and blastospores by the different species, it was observed that conidia are significantly better transmitted than blastospores. The only exception is *S. feltiae*, which still transmit a significant amount of blastospores to the centre part of the glass tubes, although further spread is low.

S. feltiae especially transmit a considerable number of conidia to the centre part, though transmission here is also significant in the other two nematode species.

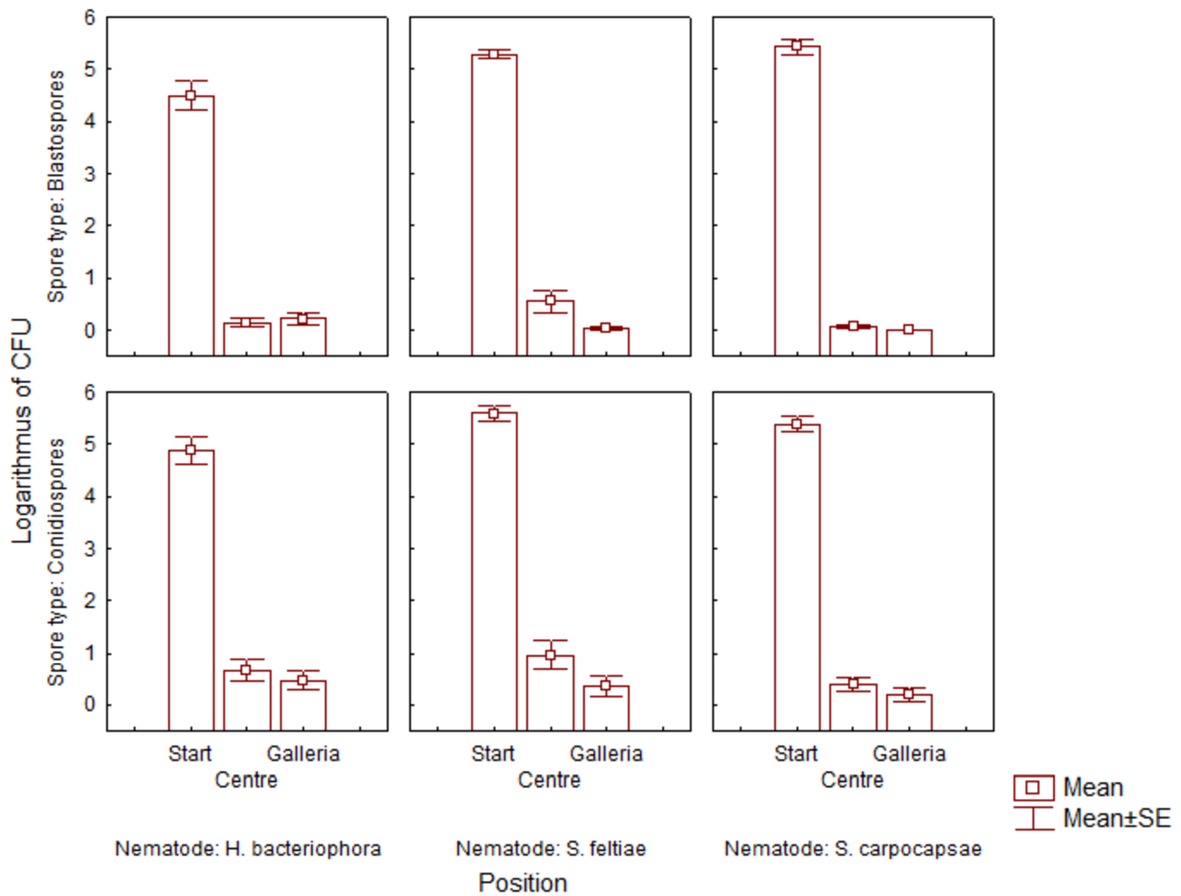

Figure 8: Logarithmically transformed number of Colony forming units (CFU) with mean (± SE) per spore type. Number of CFUs per part of the glass tube is given in individual columns (Start, Centre, G. mellonella larvae) for each of the three nematode species used.

Moreover, the presence of a 2nd stage cuticle was found to be of great importance for spore dispersal in this experiment, regardless of the nematode species. In particular, the spore dissemination seems to be significantly increased ($p<0.05$) if nematodes are en-sheathed (i.e., possess their 2nd stage cuticle), whereas de-sheathed nematodes transmit noticeably less or no CFUs. Nematodes were de-sheathed according to the process stated in the Methodology.

The *S. feltiae* strain with its 2nd stage cuticle intact was recorded to be the best transmitters for spores, in particular to the centre part of the glass tube.
En-sheathed *H. bacteriophora* HB221 showed a lower number of CFU in the first part (start), compared to *S. feltiae* and *S. carpocapsae*. However, a higher amount of these spores was transported to the end of the glass tube compared to the other species.
Additionally, analysis indicates a generally lower amount of CFU isolated from the first part of the glass tube when ex-sheathed nematodes were used. The only exception here is *H. bacteriophora*, where the opposite seems to hold true.

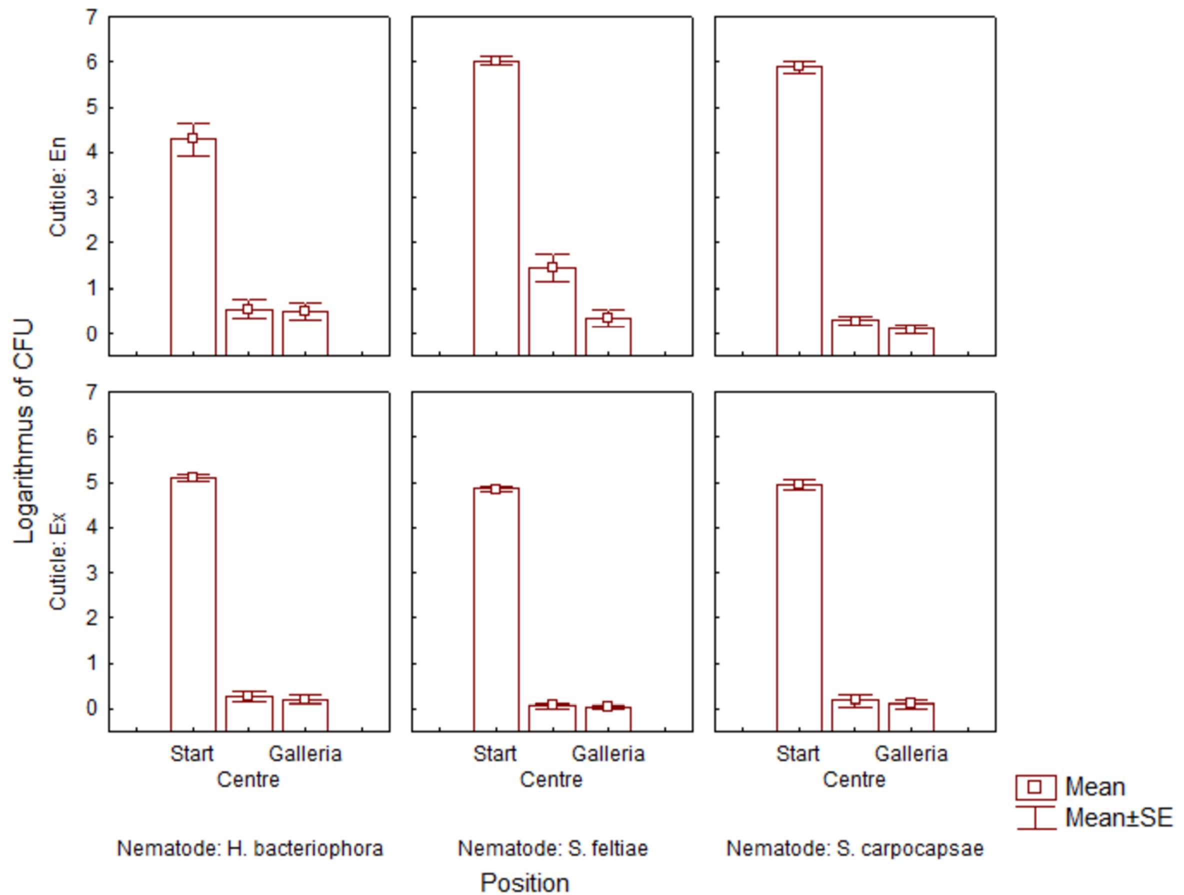

Figure 9: Logarithmically transformed number of Colony forming units (CFU) with mean (± SE) for en- and ex-sheathed nematodes. Number of CFUs per part of the glass tube is given in individual columns (Start, Centre, *G. mellonella* larvae) for each of the three nematode species used.

4.2 Soil column experiment

Statistical analysis of the observed results (see Table 2) clearly indicates that the number of spores transmitted by nematodes is significantly ($p<0.001$) influenced by the application medium, presence of the 2nd stage cuticle, nematode species, and the presence of nematodes. However, in this experiment it was found that the spore type has no significant ($p<0.05$) effects on the dissemination of spores.

Table 2: Complete results of statistical analysis (GLM) for the Soil Column Experiment. Red colour indicates statistically significant result ($p < 0.05$).

	d. f.	F	p
(1) Application medium	2	19.499	<0.001
(2) Presence of 2nd stage cuticle	1	87.271	<0.001
(3) Nematode species	2	91.544	<0.001
(4) Presence of nematodes	2	292.251	<0.001
(5) Spore type	1	0.357	0.550
1*2	2	14.446	<0.001
1*3	4	16.339	<0.001
2*3	2	10.125	<0.001
1*4	4	11.390	<0.001
2*4	2	49.980	<0.001
3*4	4	87.690	<0.001
1*5	2	0.875	0.417
2*5	1	0.703	0.402
3*5	2	6.598	0.001
4*5	2	0.089	0.915
1*2*3	4	1.602	0.172
1*2*4	4	8.716	<0.001
1*3*4	8	15.228	<0.001
2*3*4	4	10.441	<0.001
1*2*5	2	5.756	0.003
1*3*5	4	3.574	0.007
2*3*5	2	0.250	0.779
1*4*5	4	3.554	0.007
2*4*5	2	4.006	0.019
3*4*5	4	6.029	<0.001
1*2*3*4	8	1.929	0.053
1*2*3*5	4	2.682	0.030
1*2*4*5	4	4.903	<0.001
1*3*4*5	8	3.627	<0.001
2*3*4*5	4	0.484	0.747
1*2*3*4*5	8	2.873	0.004
Error	972		

Based on the analyses it was concluded that the *S. carpocapsae* strain 1343 is the most effective nematode species in spore dissemination of both conidia and blastospores. The effect of this nematode species on dissemination was found to be significantly higher ($p<0.05$) than in the case of the *S. feltiae* strain NFUST and the *H. bacteriophora* strain HB221. No meaningful differences were recorded between *S. feltiae* and *H. bacteriophora*.

Furthermore, the presence of the second stage cuticle was confirmed to be a crucial factor in spore dissemination. In general, analysis shows that nematode species transmitted significantly more ($p<0.05$) spores when en-sheathed than de-sheathed. Nematodes that were de-sheathed, in accordance with the procedure mentioned in the Methodology, disseminated very low numbers or no CFUs at all. This effect was most apparent in the case of the *S. carpocapsae* strain 1343 where results considerably differ ($p<0.05$) from other nematodes.

Another factor that significantly influenced ($p<0.001$) the efficacy of spore dissemination is the medium that was used for spore and nematode application. The effect is mainly visible in en-sheathed IJs that were applied together with spores and sunflower seed oil. According to a post-hoc test, oil supports the dissemination of spores significantly more ($p<0.05$) than liquid alginate or water. Effects were considerable mainly in conidia but also in blastospores. It was found that alginate gel significantly enhanced dissemination of spores only in combination with *S. carpocapsae*. In this case, the results significantly exceed even the oil ($p<0.05$) according to a Tukey post-hoc test.
In case of ex-sheathed IJs the effect of different media was negligible and only liquid alginate gel in combination with *S. carpocapsae* showed a significant ($p<0.05$) difference, namely via a higher transmission rate, than other combinations.

Generally, it was demonstrated in this experiment that conidia are disseminated better than blastospores; the difference between these two types of spores is clearly significant ($p<0.05$). The experiment further showed that conidia were disseminated more by both en- and ex-sheathed IJs of all nematode species.

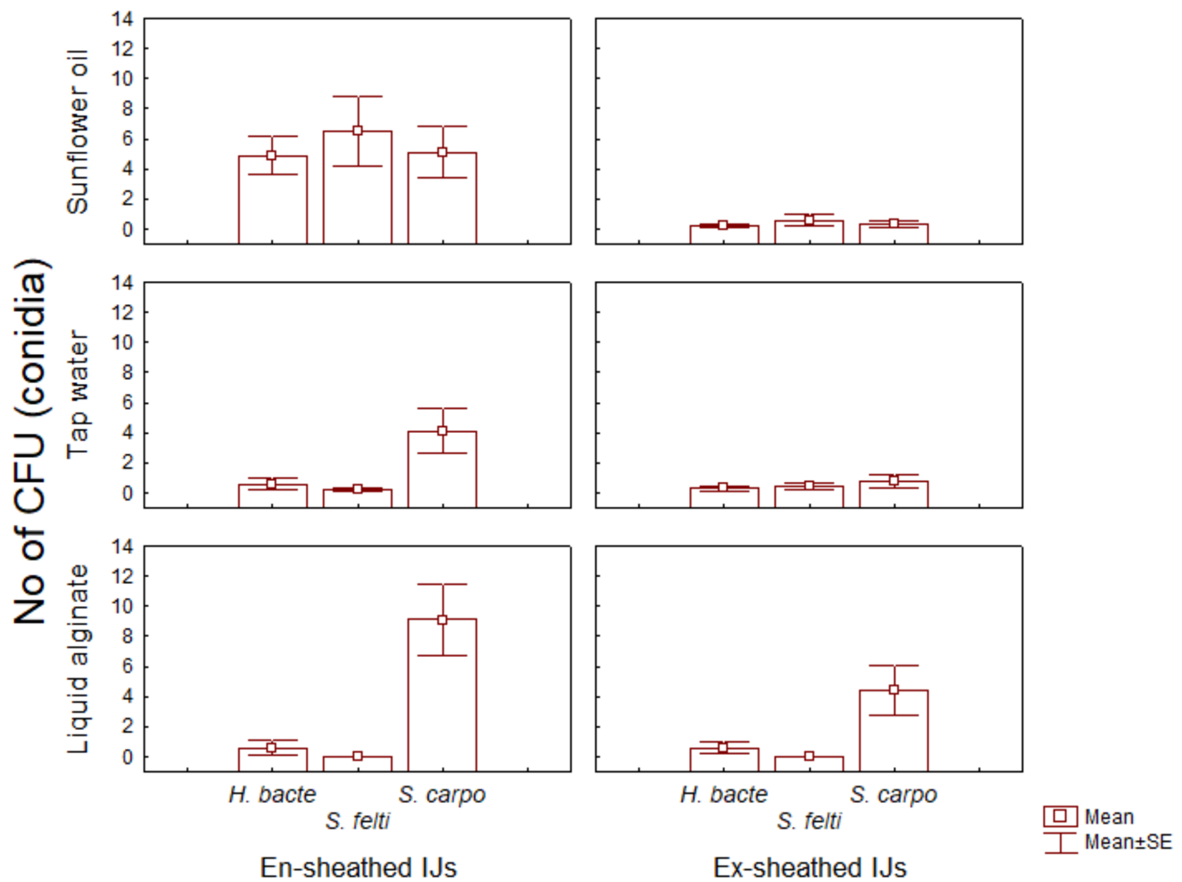

Figure 10: Number of Colony forming units (CFU) with mean (± SE) for application of conidiospores in combination with en- or ex-sheathed Infective Juveniles (IJs) of three nematode species to Soil Columns. Graphs given for different media (Sunflower oil, Tap water, Liquid alginate) used in combination with nematodes and fungus.

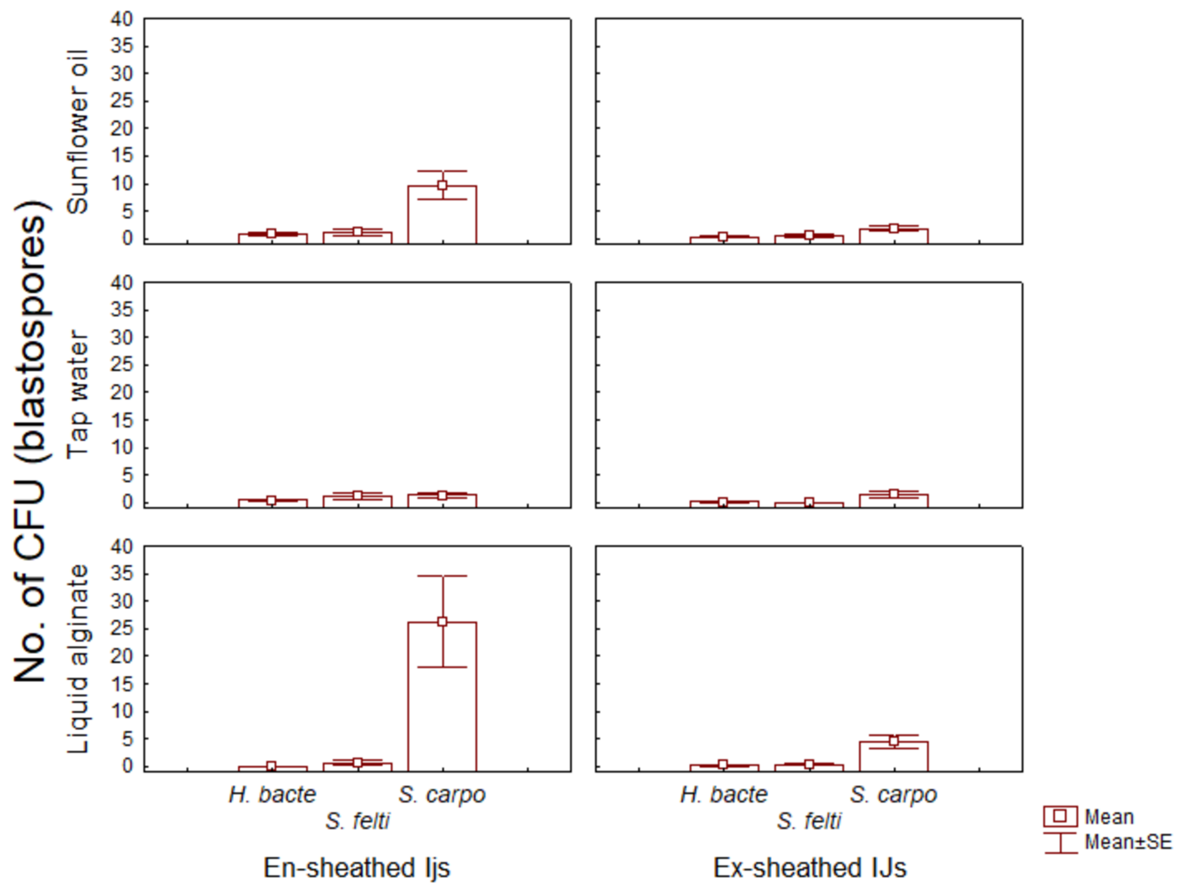

Figure 11: Number of Colony forming units (CFU) with mean (± SE) for application of conidiospores in combination with en- or ex-sheathed Infective Juveniles (IJs) of three nematode species to Soil Columns. Graphs given for different media (Sunflower oil, Tap wate water, Liquid alginate) used in combination with nematodes and fungus.

5. Discussion

The main aim of this thesis was to evaluate the impact of foraging behaviour of different nematode species on the dissemination of *C. fumosorosea* conidia and blastospores. The three different nematode strains employed in these experiments were chosen specifically to compare all three foraging strategies, namely cruiser, ambusher, intermediary and their effect. Additionally, a focus was put on the importance of the nematode's 2nd stage cuticle and its role in spore dissemination. To achieve insights, experiments were conducted with en- and ex-sheathed nematodes. Lastly, methods to enhance spore dissemination were explored, specifically by testing various adhesives which were used as an application medium.

5.1 Glass tube Experiment

The main intention of the glass tube experiment was to gain understanding of the influence of nematode foraging behaviour on spore dissemination. Generally, it was found that the application of nematodes in conjunction with fungal spores enhances their dispersal through the soil environment of the glass tube. Increased transmission, in comparison to the control with only spore application, was found with all three nematode strains. *Steinernema carpocapsae* overall exhibited the lowest number of CFUs transmitted, which may be attributed to their ambushing behaviour. Since ambushers are mainly found near the soil surface and wait for hosts to pass by [17] [18], it can be assumed that this behaviour plays a role in their lower ability to disperse spores in this experimental design.

On the other hand, *H. bacteriophora* were discovered to be best at transferring spores over longer distances, as illustrated by the highest yield of CFUs in the third part of the glass tubes. *H. bacteriophora*'s capability to disseminate spores even far down the glass tubes occurs due to their cruising behaviour. Since cruisers are very active and don't await host cues, they can be found throughout the soil profile [18], which is in accordance with the observations mentioned here.

S. feltiae show an intermediary foraging behaviour that lies between ambusher and cruiser; therefore, they are still active, but also remain stationary when no potential hosts are close by. [4] Within this experiment, this behaviour in fact translates to the strain dispersing the highest number of CFUs extracted from soil of the centre part of the glass tubes. Though, dispersal to the third part was found to be lower than that of *H. bacteriophora*, yet still higher than *S. carpocapsae*.

Analysis of the experimental data also yielded information about the spore type that is best transmitted. It was concluded that conidia are generally better dispersed through a soil environment with the aid of nematodes than blastospores. The general trends regarding number of CFUs corresponding to nematode strain described before still hold true when solely examining transmission of spore type. Reasons as to why conidia are better dispersed are not yet determined since research in this direction is still lacking, however their tolerance against environmental stresses as well as their hydrophobicity could be of importance. [34] [58]

Another important finding is the significance of the nematode's 2nd stage cuticle for dissemination of fungal spores. Data clearly shows that nematodes, regardless of strain or foraging behaviour, disperse less spores if they have been stripped of their 2nd stage cuticle. A nematode's 2nd stage sheath is commonly more corrugated than the 3rd stage sheath, which may explain why spores are able to better adhere to the 2nd stage cuticle. [70]

In general, the method used in this experiment still leaves room for improvement and alterations. For example, the wetness of the soil or usage of sterilised or unsterilised soil may influence dispersal rates.

5.2 Soil column experiment

The second experiment's focus was the evaluation of different adhesives and how they influence spore dissemination. Furthermore, hypothesis already explored in the first experimental series were assessed here as well.

Observations about spore transmission, already stated in the glass tube experiment, were confirmed in the soil column experiment. Namely, conidia were transported better and at a significantly higher rate than blastospores. Surprisingly *S. carpocapsae* were deemed to be the best dispersers of both spore types, which may be due to their ambusher foraging strategy being better suited for the experimental design. [17], [18] This contrasts with the previous experiment where the strain performed considerably worse than both *S. feltiae* and *H. bacteriophora*. The difference could be explained by the diverse soil conditions that were simulated in the two experiments. While the glass tube experiment better replicates the conditions deep within the soil, the soil column experiment is more appropriate for reproducing the conditions on the soil surface. As mentioned previously, nematodes

employing the ambushing strategy, like *S. carpocapsae*, are predominantly found near the soil surface where they await hosts. [17]
Therefore, the soil column experiment appears to be better suited to the natural foraging behaviour of *S. carpocasae*. This aligns with the findings regarding increased spore dissemination of this species in the second experiment.

Additionally, the 2nd stage cuticle's effect was once again confirmed through this experiment. Ex-sheathed nematodes, irrespective of strain, transmitted only very low numbers of spores; especially blastospore dispersal decreased significantly. Both experiments therefore point to the sheath as a crucial component for fungal spore transmission that should not be overlooked, particularly in pest management where spore dispersal enhancement is desired. [70] Nevertheless, more research should be conducted to further examine the underlying mechanism of spore adhesion to nematode's 2nd stage cuticles.

Lastly, the novel approach of using adhesives to enhance spore dissemination yielded promising results. Specifically sunflower seed oil applied as a medium with en-sheathed IJs seems to noticeably increase the number of CFUs detected for all three nematode species. It's advantage mainly presented itself in the dissemination of conidia, since it appears that blastospore transmission is barely influenced by adhesives and largely stays low. A possible explanation for this is the hydrophilicity that presents a main characteristic of blastospores. [58] Moreover, blastospores have a lower tendency to withstand environmental stresses, especially when compared to conidia, therefore possibly lowering dispersal rates. [59] Alternatively, blastospores may simply not be able to adhere to nematode's 2nd stage cuticle as well as conidia.

Tap water has been determined to be by far the weakest medium when compared to sunflower seed oil and liquid alginate. Even when conidia are applied with en-sheathed IJs only low dispersal rates were recorded, except for *S. carpocapsae* which still yielded an acceptable, albeit lower rate of spore distribution. Unexpectedly, the combination of *S. carpocapsae* with liquid alginate for the transmission of conidia exhibited the highest number of CFUs recorded within the scope of this experiment.

Overall, the use of adhesives has the potential to greatly influence the efficacy of spore dispersal by nematodes and may present an interesting opportunity for the use in integrated pest management and biocontrol. Sunflower seed oil appears to yield the best results irrespective of nematode strain and therefore might be the most promising candidate for conducting supplementary research and further improve spore dispersal.

6. Conclusion

The principal intention of this research was the evaluation of nematode foraging behaviour on conidia and blastospore dissemination in *C. fumosorosea*, assessing the importance of the nematode's 2nd stage cuticle on dissemination, as well as proposing an enhancement method for the dispersal via adhesives.

Experimental results show an effective enhancement of spore transmission through nematodes and provided insights into the role of different foraging behaviours on spore dispersion. It was found that *H. bacteriophora* and *S. feltiae* perform better in experimental designs that simulate conditions deep within the soil, while the performance of *S. carpocapsae* is superior when the soil conditions mimic those of soil surfaces.
Furthermore, it was shown that the nematode's 2nd stage cuticle is of high importance for effective spore dispersal since results indicate that nematodes transport significantly less spores without it.

Experiments with adhesives revealed that they provide a noteworthy new approach for enhancing spore transmission. The data further suggests that sunflower seed oil delivers the best dispersion rates irrespective of the nematode species with which it is applied. However, a particularly potent combination was discovered to be *S. carpocapsae* with conidia and liquid alginate as an adhesive, achieving overall highest rates of spore transmission. Nevertheless, the possibility of it being an outlier cannot be ruled out and since only *S. carpocapsae* showed a significant increase in spore dissemination when applied in conjunction with liquid alginate, it cannot be universally recommended as an adhesive. Sunflower seed oil in contrast significantly increased spore transmission in all tested nematode species and was therefore determined to be the strongest contender for use in future research.

Based on these findings, possible future research may focus on improving or changing soil conditions within the experimental apparatus to further explore the environmental impacts on spore dispersal. Supplementary examinations of the exact mechanism of spore adhesion to the 2nd stage cuticle would be of importance, to gain a better understanding of spore transmission in general.

Additionally, a focus on other possible adhesives as well as exploring sunflower seed oil and liquid alginate in combination with different nematode strains could be of interest.

7. References

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