Trends in Microbiology

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Forum

# Small-secreted proteins as virulence factors in nematode-trapping fungi

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**Nematode-trapping fungi (NTF), such as *Arthrobotrys flagrans* (*Duddingtonia flagrans*), are soil-borne fungi able to form adhesive trapping networks to attract and catch nematodes. In this forum piece we highlight some of their most fascinating features with a special focus on the role of small-secreted proteins in the predatory interaction.**

## Introduction

Filamentous fungi are key components of the soil microbiome. Many of them develop as saprotrophs, decomposing organic matter, whereas others are plant pathogens that feed on living plants. In addition, a large group are mutualistic symbionts and associate with more than 80% of all land plants. The NTF are a fascinating group of soil filamentous fungi that can switch their lifestyle from saprotrophic to predatory in the presence of nematodes, but only upon nutrient starvation (Figure 1A,B). These fungi have developed an arsenal of species-specific trapping devices, including adhesive knobs, sticks, ring-like adhesive traps, and constricting rings that aid them in hunting their prey [1].

NTF have a potential as biocontrol agents. Nematodes often cause problems in feedstock or as plant pathogens and account for annual losses of up to 120 US$ billion. NTF chlamydospores, applied as pellets to feed sheep, resist the intestinal passage and germinate on dung – where they can catch nematodes, thereby reducing the population in the field. There is also a potential to control plant-pathogenic nematodes. Besides the application of NTF as biocontrol agents, they may serve as biofertilizers. There is evidence that *A. flagrans* interacts with tomato roots, improving their phosphate nutrition and increasing their resistance to pathogenic microorganisms [2]. Hence, *A. flagrans* appears as a promising organism for sustainable agricultural practices, controlling pathogens, and promoting plant growth.

## Trap initiation requires interkingdom communication

NTF such as *A. flagrans* and *Arthrobotrys oligospora* use volatile molecules to lure nematodes into fungal colonies (Figure 1B). In the absence of nematodes, hyphae produce polyketide-derived arthrosporols that inhibit trap formation [3]. If nematodes are present, they are recognized because of nematode pheromones, the ascarosides [4]. Fungal sensing of ascarosides leads to downregulation of arthrosporol synthesis, and hence, initiation of trap formation [5]. The system resembles quorum sensing and guarantees that trap formation is initiated only if sufficient nematodes are present (Figure 1B). The interconnection of the signal transduction processes to other signaling cascades and cellular processes remains to be discovered. Interestingly, the predatory stage of NTF can be highjacked by some bacteria – which release nutritional cues, such as urea, that induce trap formation, thereby reducing the number of their nematode predators [6].

## Ring closure during trap formation requires intercellular communication

Ring-like structures are very unusual, not only in fungi but in biological systems in general, and their formation likely requires sophisticated rearrangements of the cytoskeleton and the growth machinery (Figure 1C) [7]. A cell–cell dialogue allows the growing fungal tip of a developing trap to communicate with the basal hypha, ensuring ring closure (Figure 1D) [8]. The system resembles the cell–cell communication model in *Neurospora crassa*, involving intercellular and intracellular signaling with the involvement of a MAP kinase module and the STRIPAK signaling complex [7]. However, the nature of the signal or its receptor(s), as well as the detailed intracellular signaling, has not been elucidated in any fungal system yet.

## Small-secreted proteins as novel virulence factors in fungal–worm interactions

Once a worm is trapped, hyphae penetrate into the body and colonize the entire animal. A role for lytic enzymes appears obvious, and some of them have been identified and characterized. However, the *A. flagrans* secretome comprises, in addition, more than 200 small-secreted proteins (SSPs) without similarity to known enzymes or to any other proteins [9]. In many pathogenic and symbiotic plant–fungal or plant–bacterial interactions, microbial SSPs interact with host targets to manipulate the cell program of the host [10,11]. As an example, *Candida albicans* uses a small peptide (candidalysin) to form pores in human target cells [12]. Most SSPs characterized in plant–microbial interactions contribute to prevent or to stop the defense reactions of the plant towards the microbe, but others target plant metabolic processes serving to nourish the microbe [13]. Sometimes one effector may also have different host-cellular targets and affect several processes [14]. Hence, it is clear that, although SSPs may have different cellular modes of action, their role is to control the host cell. Whether such SSPs are involved in the interaction between NTF and their prey, or whether the fungus just kills the worm with a potent toxin and subsequently digests the biomass, is an open question which is currently under investigation.

If SSPs play a role, one could speculate that some could have a cytolytic function

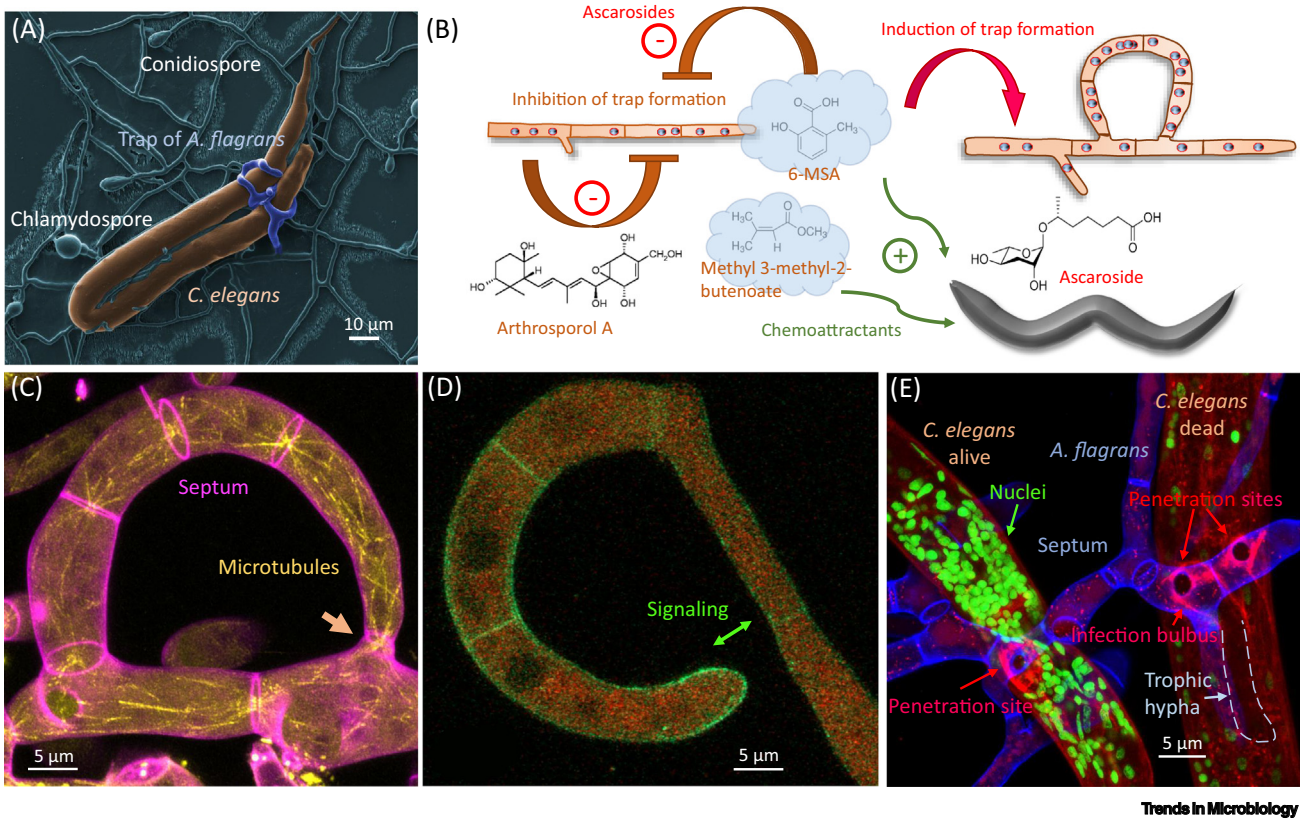


Figure 1. The predatory lifestyle of *Arthrobotrys flagrans* (*Duddingtonia flagrans*). (A) Scanning electron micrograph of trapped *Caenorhabditis elegans* (colored in brown) and the fungus *A. flagrans*. Traps are colored in blue. Conidia and chlamydospores are also visible [9]. (B) Arthrosporols and 6-MSA inhibit trap formation in *A. flagrans*. 6-MSA and other small volatiles, such as methyl-3-methyl-2-butenoate, attract *C. elegans*, and nematode-derived ascarosides inhibit the production of the trap-inhibiting molecules, thereby inducing trap formation [5]. Another prerequisite for the transition from saprotrophic growth to a predatory lifestyle is nutrient starvation. (C) Trap morphology and the microtubule cytoskeleton. Cell walls were stained with calcofluor (pink), and microtubules were labeled with a fluorescent protein (yellow). 3-D image taken from [7]. (D) Hyphal fusion during trap formation involves a ping-pong signaling mechanism. One of the signaling proteins was expressed as a green fluorescent protein (GFP)-fusion protein and accumulates at the growing tip. A few seconds later it will highlight the membrane of the receiving hypha. The chemical nature of the signaling compound is still unknown [8]. (E) Late stage of the infection with two *C. elegans* caught by the fungus. Nuclei of the nematode were stained with GFP. The left nematode is still alive, and its nuclei are highly fluorescent, whereas the right nematode is dead, and its nuclei do not glow anymore. Fungal cell walls are stained in blue, and the pink color shows the virulence factor CyrA as an mCherry-fusion protein; it labels the penetration site and the infection bulbus underneath the hypodermis [15]. Abbreviation: 6-MSA, 6-methyl salicylic acid.

similar to candidalysin, aiding in killing the prey, even if lytic enzymes seem to be most important. Currently, only two SSPs of *A. flagrans* have been identified and characterized to some extent. One of them contains a nuclear localization signal that targets the protein to nuclei when heterologously expressed in *Caenorhabditis elegans,* suggesting a putative function for this fungal protein in the nucleus of worm cells [9]. The second SSP, CyrA, caused a reduction in the *C. elegans* lifespan when expressed in the worm [15]. A fungal strain lacking CyrA was less virulent. The protein is specifically produced in traps and appears to be secreted at the penetration site and at a bulbus-like structure produced after the hypha has entered the worm's body (Figure 1E). Later, during growth of the trophic hyphae, emerging from the bulbus and extending within the worm's body, CyrA is no longer visible. These results indicate a specific action of CyrA shortly after penetration and suggest that the bulbus might be a specific structure for secretion of CyrA and perhaps of other SSPs. This is reminiscent of the biotrophic interfacial complex (BIC) observed during the interaction between the plant pathogen *Magnaporthe oryzae* and rice plants. The BIC is a membrane-rich, plant-derived structure and has been associated with the translocation of fungal cytoplasmic effectors into the rice cell [14]. Despite some morphological similarities between the BIC and the bulbous hypha of *A. flagrans*, and the possible conservation of their functions for secretion of virulence factors, there are major differences. Whereas the BIC is a structure at the interface between fungal and plant cell

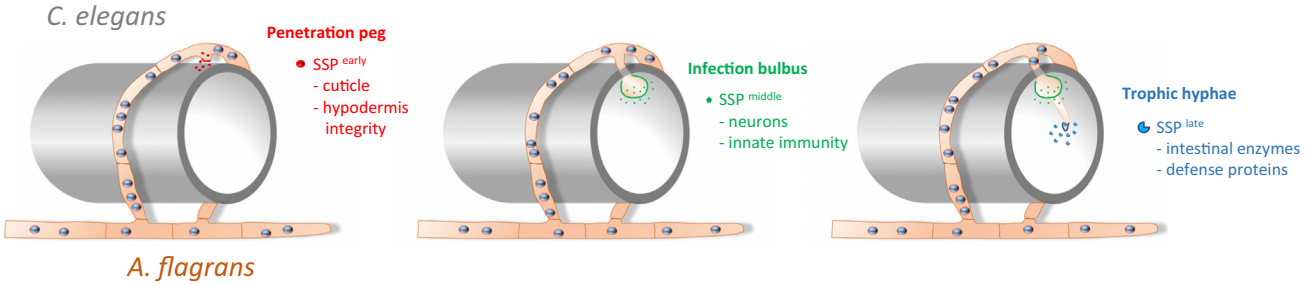


Figure 2. Scheme for the penetration process. The fungal small-secreted proteins (SSPs) may be divided, according to their time of action, into early-, middle-, and late-stage SSPs. Putative targets of the virulence factors are named. Species: *A. flagrans, Arthrobotrys flagrans; C. elegans, Caenorhabditis elegans.*

and of plant origin, the NTF bulbus appears like a swollen hypha, and it is probably of pure fungal origin.

Taken together, these novel data about SSPs in *A. flagrans* suggest sophisticated interaction of NTF and nematodes and foster the hypothesis that SSPs could be involved at different stages of the predatory process. This way, SSPs at initial stages could facilitate the entry of hyphae into the worm's body by destabilizing the cuticle. Then, SSPs could be involved in tranquilizing or killing the prey by acting on specific neurons or by inactivating *C. elegans* defense proteins. In later stages, when hyphae proliferate in the worm's body, SSPs could target digestive enzymes of *C. elegans* – in a manner similar to that of Kunitz protein inhibitor proteins from legumes that modulate protease activities. In conclusion, we propose to categorize SSPs into early, middle, and late factors (Figure 2). It will be the challenge of future research to identify their molecular targets in *C. elegans.*

## Concluding remarks

The interaction between NTF and nematodes is characterized by a cascade of signal-exchange processes between fungal hyphae and also between fungi and nematodes. The sophisticated communication reflects million of years of coevolution, as evidenced from fossils older than 400 million years. Recent results suggest that the predatory lifestyle of NTF is not only a quick killing, and subsequent digestion of the animal, but that a large number of small-secreted fungal proteins are used to successfully overcome nematode defense reactions. The establishment of molecular and cell biological tools in the two models *A. oligospora* and *A. flagrans* in recent years provides an extraordinary opportunity to decipher the secrets of their underground communication and chemical interaction with worms. Hopefully, the study of the fungal–nematode interaction will also help us to better understand other fungal–animal interactions, including fungal–human interactions. Moreover, further research on NTF might contribute to improve their application as biocontrol agents against plant- and animal-pathogenic nematodes, promoting a more sustainable agriculture.

## Declaration of interests

No interests are declared.

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