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Review

Fatal attraction: How *Phytophthora* zoospores find their host

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ABSTRACT

Oomycete plant pathogens, such as *Phytophthora* and *Pythium* species produce motile dispersal agents called zoospores that actively target host plants. Zoospores are exceptional in their ability to display taxis to chemical, electrical and physical cues to navigate the phyllosphere and reach stomata, wound sites and roots. Many components of root exudates have been shown attractive or repulsive to zoospores. Although some components possess very strong attractiveness, it seems that especially the mix of components exuded by the primary host is most attractive to zoospores. Zoospores actively approach attractants with swimming behaviour reminiscent of other microswimmers. To achieve a unified description of zoospore behaviour when sensing an attractant, we propose the following terms for the successive stages of the homing response: reorientation, approaching, retention and settling. How zoospores sense and process attractants is poorly understood but likely involves signal perception via cell surface receptors. Since zoospores are important for infection, undermining their activity by luring attractants or blocking receptors seem promising strategies for disease control.

1. Introduction

Virtually all oomycete plant pathogens make use of zoospores, motile dispersal agents which are able to actively target host plants. Its motility contrasts the oomycete zoospore to the immotile spores of fungal plant pathogens, pathogens which although morphologically similar to the oomycetes, phylogenetically are utterly distinct [1]. Agriculturally and ecologically devastating oomycetes include species of the genera *Pythium* [2], major agents of seedling damping-off, and *Phytophthora*, which cause a variety of devastating diseases on many different types of plants of all ages, ranging from seedlings of annual vegetables to fully developed forest trees [3,4]. *Phytophthora* means plant destroyer, and with good reason. At present over 190 species have been described [5]. The majority are soilborne pathogens that cause mainly root and stem rots and necrotic lesions on woody roots while the airborne *Phytophthora* species cause blights of the foliage, young twigs and fruit, shoot dieback and bleeding bark lesions. Some well-known examples are *Phytophthora infestans*, the Irish potato famine pathogen causing late blight [6], *Phytophthora sojae*, the soybean root and stem rot pathogen [7], and *Phytophthora ramorum* that causes Sudden Oak Death and is pathogenic on many woody plants and trees [8].

Zoospores use their motility to actively target their host, accumulating heavily on roots, stomates and wounds (Fig. 1B, C). This behaviour, known as 'homing', implies there are cues that guide zoospores to their host, and that zoospores are equipped to recognize these. Previous reviews addressed the roles of zoospores in the infection cycle [9], the cell biology of zoospores [10] and soil-zoospore interactions [11] . This review focusses on the capacity of zoospores to respond to environmental cues. We first summarize the known tactic responses of zoospores and in particular the role of chemotaxis. We then elaborate on how zoospores locate their specific hosts, and address the questions where cues originate in the rhizo- and phyllosphere and how zoospores perceive these cues. Finally, we reflect on how understanding of zoospore homing behaviour can be exploited for innovative crop protection strategies.

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2. Zoospores initiate the infection cycle

For virtually all oomycete plant pathogens, zoospore taxis is the prelude to a successful infection. The zoospore docks at a potential host surface, retracts its flagella, exudes adhesins to firmly attach and synthesizes a cell wall. The cysts rapidly germinate and emerging germ tubes start to explore the plant surface in search for a suitable spot to invade. Some species enter the host via stomata, others penetrate the cuticle and grow further in the epidermal cell underneath by applying pressure. Recent studies in *Phytophthora palmivora*, *P. capsici* and *P. infestans* have shown that in these pathogens germ tubes emerging from cysts adhere to the plant surface and form a hyphal swelling at the tip, thereby generating mechanical forces that slice the surface under an oblique angle [12]. This 'naifu invasion' mechanism differs from the appressorial invasion mechanism in fungi that requires melanin and septins, components lacking in oomycetes [13]. During penetration the hyphal tip acts as microscopic knife whose sharpness is mediated by an actin-based mechanostat [14]. Although *Phytophthora* species, many being hemi-biotrophs and needing living host tissue to survive, definitively need a mechanism to gain access to the plant, it is unclear whether necrotrophic *Pythium* species, prospering on dead cells, do so as well. After invasion (hemi-)biotrophs colonize the inner cell layers where the hyphae grow profusely intracellular while forming haustoria that grow into the cells. Haustoria facilitate uptake of nutrients from the host and delivery of effectors into plant cells. Effectors promote the virulence of the pathogen. They suppress host immunity thereby paving the way for the pathogen to proliferate and colonize the host, culminating in sporulating lesions producing numerous sporangiospores. Some oomycetes first disperse sporangiospores by wind and water, which then can germinate directly to infect hosts. Under the right conditions however, i.e. high humidity and/or low temperatures, zoosporogenesis is initiated: the sporangiospore, now termed zoosporangium, releases a multitude of zoospores to start the infection cycle all over again.

3. Zoospores hallmark an aqueous habitat

Historically, oomycetes are known as 'water moulds'; 'mould' from their shared morphology with fungi, and 'water' for their prevalence in aqueous environments. In line with this, oomycete plant diseases skyrocket during persistent precipitation and on saturated soils and their zoospores are accordingly equipped to deal with such soggy conditions. Oomycete zoospores are microswimmers, which is an overarching term for single cells propelled by one or several flagella. They have a kidney-shaped wall-less body with two flagella inserted in a ventral groove (Fig. 1A). Oomycetes share their flagellar set-up with other heterokonts, such as the brown algae: one smooth posterior (whiplash) flagellum and one 'hairy' anterior (tinsel) flagellum covered by tripartite tubular hairs called mastigonemes (Fig. 1A) [15,16].

Through coordinated beating and mastigoneme-dependent thrust reversal of the anterior flagellum [16], zoospores can actively traverse aqueous environments. While swimming, zoospores rotate along their axis; in reality the trajectory is helical [16–19], but when projected it

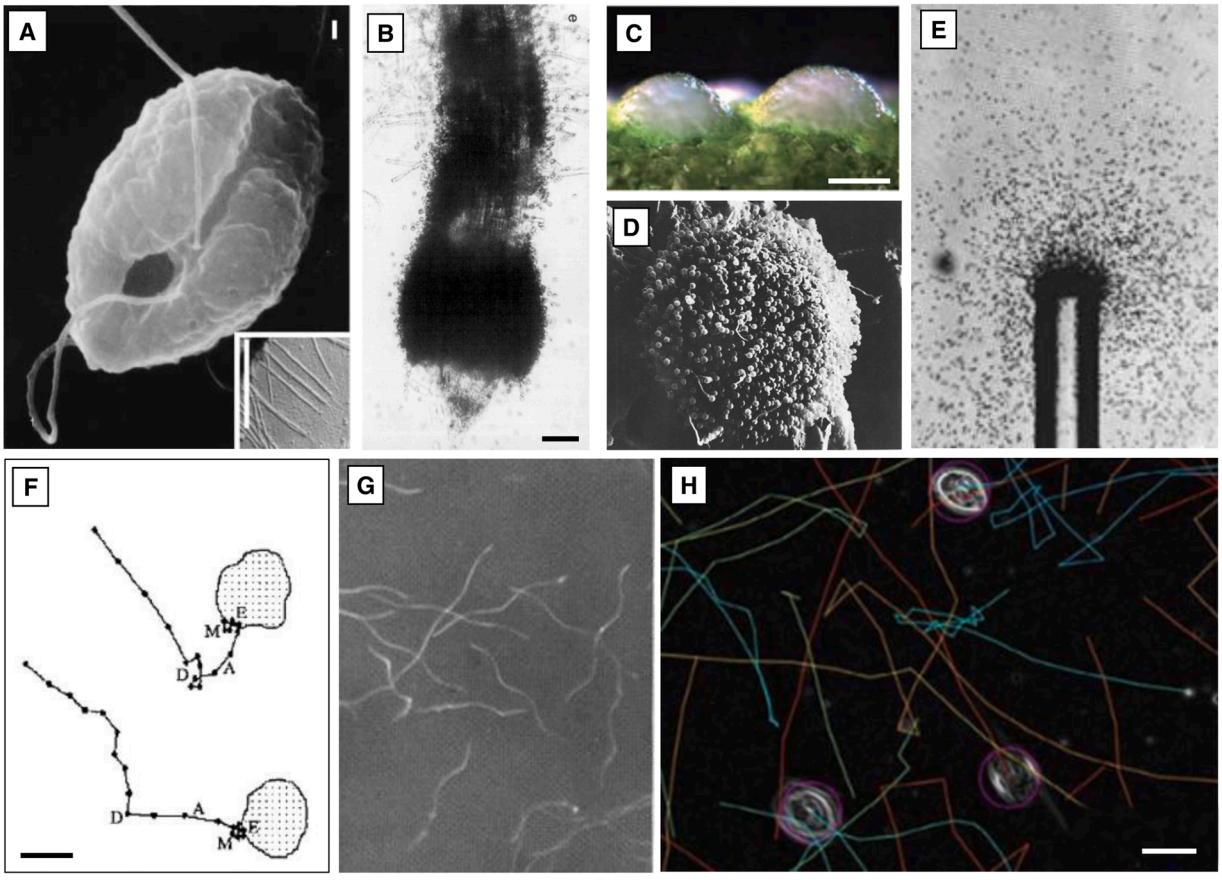

Fig. 1. Zoospore biology and behaviour during taxis. A| *P. cinnamomi* zoospore with typical kidney-shaped body and two flagella inserted in the ventral groove. Inset shows detail of mastigonemes on the anterior flagella. Reproduced from [104] B| *P. palmivora* zoospores aggregating at the plant root elongation zone. Reproduced from [70] C| *P. parasitica* zoospores aggregating to puncture wounds on plant leaf, forming biofilms. Reproduced from [69] D| Autotaxis of *P. drechsleri* zoospores, resulting in aggregates. Reproduced from [105] E| *Py. aphanidermatum* zoospores aggregating at *a* capillary containing pea root extract in agar. Reproduced from [42] F| Trace drawings of *P. palmivora* zoospores during autotaxis. Reproduced from [70] G| Dark-ground trace photographs of *Py. aphanidermatum* zoospore trajectories. Reproduced from [42] H| *P. parasitica* zoospores traced by automated single particle tracking. Reproduced from [11] Scalebars represent 1 μm (A), 100 μm (B, C, F) or 10 μm (H). Magnifications are 400x (D), 40x (E) and 60x (G).

appears sinusoidal (Fig. 1G). Some zoospores however are reported to not swim helically, but straight: Appiah and Gow [19] for example found *P. palmivora, P. megasperma,* and *P. infestans* zoospores to swim straight, in contrast to other studies [20,21] that reported helical swimming for the latter two. Donaldson and Deacon [22] showed that it is possible for a zoospore to display both types of movement: inhibition with the Ca^{2+} chelator EGTA caused a switch from helical to straight swimming in *Pythium aphanidermatum, Pythium catenulatum and Pythium dissotocum*.

Unobstructed, a zoospore can maintain its pathway for considerable distances [16]. The swimming runs can be interrupted by tumbles, i.e., a sudden change of direction [17–19]. Between species, zoospore swimming characteristics differ: species swim at markedly different speeds, and the amplitude used to describe their sinusoidal pathway differs as well [19,22]. Similarly, the rate of tumbling seems to differ between species; in the same experimental set up, zoospores of *Phytophthora megakarya*, *P. infestans*, and *Py. dissotocum* tumbled at alternate rates [19]. Remarkably, in that study *P. palmivora* and *Py. aphanidermatum* were reported not to tumble at all [19], whereas other studies did report them to do so [18,22]. An explanation for this discrepancy might lie in the zoospore's nature to tumble when colliding with another zoospore or a surface [17]. Hypothetically, this should lead to zoospores swimming long, straight stretches in low complexity environments with tumbling becoming more prominent as environment complexity, be it soil geometry or zoospore density, increases. In line with this hypothesis, *Phytophthora cinnamomi* zoospores were reported not to tumble in relatively spacious imaging chambers [16], whereas they did so in the more restricted environment of a capillary tube [17].

4. Zoospores actively navigate complex environments

The phytosphere as encountered by *Phytophthora* species is a far cry from the marine habitat navigated by zoospores from species in basal oomycete lineages such as the *Eurychasmales* and *Olpidiopsidales* [23,24] that parasitize algae [25]. In the rhizosphere zoospores of 'terrestrial' oomycetes are challenged with a complex geometry of channels and obstacles raising the question how they navigate to reach their hosts. Zoospores of root pathogens such as *Phytophthora cactorum*, *P. capsici* and *P. cinnamomi* maintain the ability to actively traverse complex geometries: zoospores successfully migrate through soil mimics [26,27] and complex soil types [28], where they actively target specific root zones, such as the elongation zone, and wound sites.

In the phyllosphere the environment geometry is considerably less complex, but here the discontinuity of water films brings the relevance of zoospores into question. Among the foliar pathogens there are some downy mildews, for example *Hyaloperonospora* species, that have lost flagella and disperse passively via airborne conidia [29]. Nevertheless, motility on the leaf surface does not seem useless: zoospores of foliar pathogens that retained their flagella can actively aggregate on the leaf surface, creating masses of cysts sometimes referred to as microcolonies or biofilms. Typical zoospore aggregation was observed for *P. palmivora* and *Plasmopara viticola* on stomates of oil palm leaves [30], and grapevine leaves, respectively [19], and for *Phytophthora parasitica* on wound sites on tobacco leaves [69]. Zoospores are thus very capable of actively targeting and aggregating on plant parts, but how do they make sure to end up there?

5. Zoospores show a multitude of taxes

Research on oomycete zoospore taxis sheds light on how zoospores increase their chance to encounter the roots of their hosts. Taxis is defined as an active movement of a cell or organism towards or away from a stimulus and is classified based on the nature of the stimulus. Oomycete zoospores show various types of taxis (Fig. 2). Positive rheotaxis was shown for *P. capsici* zoospores [31], allowing them to swim against a current, supposedly preventing being flushed down in the soil. Similarly, negative geotaxis of *Phytophthora nicotianea, P. cactorum* and *P. palmivora* zoospores [32] was postulated to enhance the zoospore's ability to remain close to the soil surface where rootlets are more abundant than deeper in the soil. Zoospore tumbling on collision implies they react to physical stimuli, pointing to thigmotactic behaviour [17]. Royle and Thomas [33] showed that zoospores of *Pl. viticola* targeting stomates, were not attracted to leaf-mimics, thus ruling out thigmotactic behaviour in response to the plant surface. Besides taxes of a physical nature, striking images of zoospores aggregating on the elongation zone of rootlets (Fig. 1B) suggested the plant root itself to drive zoospore aggregation. One of the cues results from electric currents produced by specific zones on the plant root. In vitro studies by Khew and Zentmyer [34] showed *Phytophthora* zoospores to be electrotactic, and Morris and Gow [18] found zoospores of different species to be specifically attracted to either the cathode or anode. Van West et al. [35] showed a relation with the preferential aggregation site on the root: the anodic apex of rye grass roots attracted *P. palmivora* zoospores, whereas the cathodic elongation zone attracted *Py. aphanidermatum* zoospores. Similarly, wounds, shown to be cathodic, repelled zoospores of *P. palmivora*, while attracting *Py. aphanidermatum* zoospores. Selective recruitment through electrotaxis therefore explains the patterning of zoospore aggregation on roots that cannot be ascribed to chemotaxis, the most intensively studied form of taxis elicited by roots [35,36].

6. Root exudates both attract and repel zoospores

Plant roots continuously release a variety of compounds in the soil.

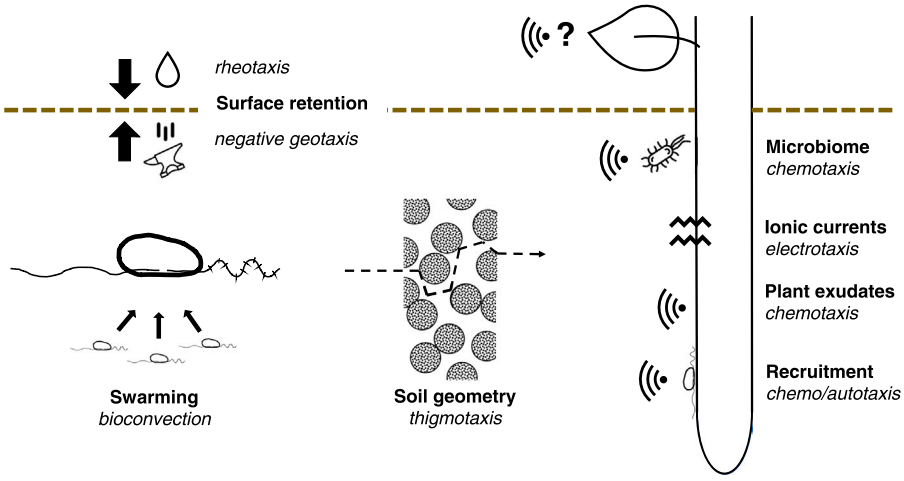

Fig. 2. Cues in the phytosphere affecting zoospore swimming behaviour. Rheotaxis and negative geotaxis retain zoospores to the upper soil layers. Swarming, possibly through bioconvection, may facilitate mass migration of zoospores. Thigmotaxis triggers soilborne zoospores to tumble and thereby facilitates navigation in the complex geometry of the soil. The plant root elicits both electrotaxis to ionic currents originating from wound sites and specific root zones, and chemotaxis to root and wound exudates. The root microbiome is a source of strong zoospore repellents and is also capable of altering the attractiveness of roots and root exudates to zoospores. Zoospores and cysts elicit autotaxis, thereby recruiting other zoospores, a process likely involving chemotaxis.

Root exudates are a cocktail of primary metabolites such as amino acids, carbohydrates, alcohols and organic acids, and secondary metabolites such as glucosinolates, hormones and flavonoids [37]. The composition of the cocktail and the relative concentrations of the metabolites therein varies per plant species and is influenced by the growth conditions, such as soil structure and composition, and abiotic stress including drought or flooding. Besides attracting beneficial microbes, root exudates also unintendedly attract parasites. Root exudates have long been hypothesized to guide the zoospore to the root, and zoospores were thus expected to display chemotaxis specifically towards root exudates from host plants [38]. Due to the initial difficulty of monitoring zoospores directly, methods to study zoospore chemotaxis have focused on analysing where zoospores end up when exposed to an attractant diffusing from a capillary tube [39] (Fig. 1E), bead [40] or agar plug [41], using the accumulation of zoospores or cysts at the source of an attractant as proxy for its potential to attract zoospores. Evidence for chemotaxis is based on findings that zoospores are attracted to their host's root exudate. Examples include *Py. aphanidermatum* zoospores strongly attracted to pea root exudate [42,43], *P. sojae* zoospores to soybean root exudate [44], *P. cinnamomi* zoospores to avocado root exudate [38] and *Aphanomyces cochlioides* zoospores to sugar beet root exudate [45]. This begged the questions: can we determine what chemical(s) in these exudates attract(s) zoospores to roots, and can we exploit that knowledge to control the disease [42]?

It turns out that not one or a few, but many root exudate components elicit chemotaxis in zoospores. A general attractiveness seems especially true for primary metabolites. For the alcohols it was shown that methanol and isopropanol attract *P. cinnamomi* zoospores [46], butanol *Py. aphanidermatum* zoospores [36] while ethanol elicits chemotaxis in many *Phytophthora* species [41,46–48]. During flooding, when plants need to protect themselves against toxic compounds accumulating during anaerobiosis, ethanol secretion is strongly increased [37], and in this regard, there might be a selective advantage for waterborne zoospores in utilizing ethanol as a homing signal. Of the organic acids iso- and valeric acid were shown to be attractive to *P. palmivora* [49] and *P. cinnamomi* [46]. In addition, Cahill and Hardham showed that *P. cinnamomi* is also attracted to several other organic acids such as ascorbic, maleic and citric acid [46], just as Cameron and Carlile showed *P. palmivora* to also be attracted to iso- and caproic acid [49]. Still, among the primary metabolites amino acids are the most studied attractants. A noteworthy variety of amino acids attracts zoospores of many *Phytophthora* [48,50] and *Pythium* species [36]. Amino acids such as alanine, aspartic acid, asparagine and glutamine have been found to attract zoospores of such a broad range of species that the term 'broad-range attractant' comes to mind [36,41,46–48,50–53]. The most striking is glutamic acid. It is capable of attracting zoospores of many root pathogens including *Phytophthora citricola, P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. palmivora* [50], *P. nicotianae* [47] and *P. sojae* [52]. Likewise, it attracts zoospores of all tested *Pythium* species: *Py. aphanidermatum* [36,39,53], *Py. catenulatum* and *Py. dissocotum* [53] and this even extends to zoospores of foliar pathogens – *P. infestans* [44] -, saprophytes -*Halophytophthora vesicula* [51]- and animal pathogens -*Saprolegnia diclina* [54]. The primary metabolites raffinose [36], hydroxyproline [52] and butanol [46] have for now only been reported once as a zoospore attractant and were tested on only one or a few species. Whether these are also 'broad-range attractants' remains to be answered.

Although often only a single concentration of the chemoattractant is tested, it is apparent from several studies that the concentration has to reach a certain threshold before the chemoattractant is able to attract zoospores, and that beyond this threshold there is a dose-dependent increase in zoospore aggregation. For example, Cahill and Hardham [46] found concentrations of 1 mM glutamic acid to strongly attract *P. cinnamomi* zoospores, whereas they were increasingly indifferent to lower concentrations. Interestingly though, at concentrations exceeding 1 mM, zoospores were repelled. These concentration dependent effects were also demonstrated for several other compounds [46]. Besides attraction or indifference, root exudate components can thus also repel zoospores, and thereby induce 'negative chemotaxis'.

Cations seem to be unanimous in repelling zoospores: Na^+, H^+ and K^+ were found to repel zoospores of *P. parasitica* [55], *P. palmivora* [56], *P. cinnamomi* [57] and *Py. aphanidermatum* [39], with the latter two also shown to be repelled by NH_4^+. Cameron and Carlile [56] subjected *P. palmivora* zoospores to a wider set of cations, including Li^+, Cs^+ and Mg^{2+}, that all induced negative chemotaxis. Besides cations, several primary metabolites have a repelling effect on zoospores. Halsall [47] reported several sugars and organic acids to repel zoospores from five *Phytophthora* species, including *P. cinnamomi*. Cahill and Hardham [46] confirmed the repellent effect of sucrose on *P. cinnamomi* and showed several other sugars to do so as well. Of the alcohols, ethanol was shown to repel zoospores of *P. nicotianae* and *P. citricola* [47] as did a range of alcohols including ethanol when tested on *Py. aphanidermatum* zoospores [36]. Of the amino acids, lysine repelled zoospores of all five *Phytophthora* species tested by Halsall [47] and likewise did so for *P. cinnamomi* [41,46] and *Py. aphanidermatum* [39]. Similarly, phenylalanine and threonine were reported to be repellent more often than not [36,46,47].

That zoospores benefit from attraction to amino acids seems logic, but what is the role of negative chemotaxis in a zoospore's homing response? Royle & Hickman [39] found some root exudate components to initially repel zoospores, but to trigger aggregation at a set distance from the capillary mouth, creating an 'arc'. They dubbed this 'arc-attraction', with the 'arc' possibly reflecting a boundary where an initial attractive component became repellent [46]. Allen & Harvey [57] argued repellence to be a simple avoidance response to prevent cell damage, e.g. due to unfavourable pH. Considering the primary metabolites however, this hypothesis seems unlikely, as in many studies it was pointed out that zoospores of different species were either repelled or attracted to a certain chemoattractant [36,47,48,50]. For example, Halsall [47] showed that for the same concentration of glutamine, *P. cinnamomi* zoospores were repelled while zoospores of *Phytophthora drechsleri* and *Phytophthora cryptogea* were attracted strongly, *P. citricola* mediocrely, and *P. nicotianae* only slightly. Now why would zoospores of different species react differentially to the same concentration of an attractant?

7. Zoospores preferentially target the host root

Even though zoospores can be attracted to both non-host and host roots [39], they are generally more strongly attracted to roots and root exudates of their own host, rather than a non-host [58]. Similarly, they are more attracted to roots of susceptible rather than resistant cultivars [59]. Each plant species secretes a unique cocktail of root exudate components and this cocktail may even vary among ecotypes or cultivars of the same species [37]. This implies that the ratios of the various components in the cocktail as well as the presence of unique components could drive host specificity by attracting zoospores of compatible pathogens. In favour of the latter is the attractiveness of secondary metabolites in root exudates. Unique attractants were identified for zoospores of several *Aphanomyces* species: indole-3-carbaldehyde from cabbage for *Aphanomyces raphani* [60], prunetin from garden pea for *Aphanomyces euteiches* [61] and cochliophilin A and N- trans--feruloyl-4–0-methyldopamine from pig weed for *A. cochlioides* [62]. Similarly, Morris and Ward [48] found that the isoflavones daidzein and genistein strongly attract zoospores of the soybean pathogen *P. sojae*, but not of *Phytophthora* and *Pythium* species non-pathogenic on soybean. Tyler et al. [63] screened compounds with structural similarity to daidzein and found 27 out of 59 to elicit attraction, particularly those with phenolic 4′- and 7-hydroxyl groups, suggesting the receptor involved to be able to bind to moieties with the same planar 3D structure as daidzein. Zhang et al. [44] showed that *P. sojae* zoospores are attracted to soybean roots and root exudates and confirmed the strong

attractiveness of daidzein and genistein. Surprisingly though, they found that daidzein and genistein concentrations in root exudate did not significantly differ between the host, soybean, and a non-host plant species, and neither between a susceptible and a resistant soybean cultivar, even though zoospores were superiorly attracted to the susceptible soybean cultivar. Other root exudate components, most notably amino acids, did differ in occurrence and concentration in the different root exudates. Interestingly, when root exudates were reconstituted in vitro by mixing the individual components, the attractiveness for zoospores dropped significantly in comparison to the sum of the attractiveness of each component individually. This implies that the various root exudate components influence each other's capacity to attract zoospores. Moreover, in the case of *P. sojae* it seems that the attractive capacity of genistein and daidzein is greatly reduced in exudate of non-hosts. This may explain why Hosseini et al. [64], found zoospores of *P. sojae* and closely related species to be attracted to non-host isoflavones. It thus seems zoospores perceive attractants in relation to other root exudate components, where individual components can have their 'attractiveness' reduced or enhanced by the presence of other root exudate components. Donaldson and Deacon [53] reported on a similar phenomenon in *Py. aphanidermatum*; they found that the attractiveness of glutamic acid was reduced in the presence of other amino acids, including glutamine, alanine and asparagine. In addition, Halsall [47] reported synergistic effects between amino acids and ethanol and sucrose, showing how the attractiveness of a root exudate can be greater than the sum of its parts. Hypothetically, root exudate synergy can thereby dictate host specificity with zoospores only being highly attracted to the precise host root exudate cocktail.

8. Zoospores influence eachother through their own exudates

In their natural habitat zoospores not only encounter host plants, they also encounter themselves. Zoospore density greatly influences infectivity, and in many species the zoospore density has to reach a certain threshold before infection takes place [65]. Zoospore behaviour in vitro is likewise affected by zoospore density. When the density is sufficiently high zoospores start swarming, with clusters of zoospores becoming so dense as to be visible with the naked eye [20,32]. Ochiai et al. [66] showed that bioconvection drives this type of behaviour, where cell geometry and motility causes zoospores to aggregate. Savory et al. [67] did not observe swarming in a relatively shallow depth and therefore it is questionable to what extent bioconvection influences zoospore behaviour in natural conditions in the phytosphere. It is however interesting to note that, theoretically, chemotaxis can attract swarming zoospores collectively, arguing for the possibility of swarm migration [67]. In favour of this are observations by Thomas and Peterson [68], who found zoospores to approach an attractant source collectively as a swarm. This swarm showed high attractive capacity of its own, thereby presumably enhancing inoculum potential. This points to zoospore autotaxis, a behaviour also described by Galiana et al. [69], who showed that aggregates of *P. parasitica* zoospores on plant wounds are initiated by a single zoospore, which then recruits a mass of zoospores to form microcolonies or biofilms (Fig. 1C, D). The microcolonies themselves possess the capacity to attract zoospores, a feature confirmed by Reid et al. [70], who showed in *P. palmivora* that aggregated encysted zoospores attract other *P. palmivora* zoospores. Similarly, Kong et al. [71] showed that a low-density zoospore suspension of *P. nicotianae* did not aggregate at host tissue, but did so when supplemented with the fluid from high-density zoospore suspensions. *P. nicotianae* zoospore exudate also induced aggregation of zoospores from several other *Phytophthora* species [72], although Reid et al. [70] found aggregates of *P. palmivora* zoospores to be unattractive to several *Pythium* species. Kong et al. [71] found Autoinducer-2, a signalling molecule involved in bacterial quorum sensing, to be present in zoospore exudates, although they did not find it to induce zoospore aggregation in their experiments. Galiana et al. [69] identified cyclic AMP as candidate for inducing autotaxis: it is present in *P. parasitica* biofilms and able to attract zoospores in vitro. Jiang et al. [73] did identify leucine in zoospore exudates to be able to restore cyst germination in low-density zoospore suspensions of *Phytophthora erythroseptica.* Thus, zoospores attract and strongly influence each other's behaviour, likely to increase disease pressure. Our knowledge on how they do so is preliminary at best, and therefore it is worth to further explore the zoospore autotaxis mechanisms.

9. Zoospores actively approach attractants

Root exudates only diffuse several millimetres away from the plant root due to the high turnover rate imposed by the soil microbiome [74]. Although compounds that are attractive at nanomolar levels, like daidzein, might diffuse into the soil at physiologically relevant concentrations, it is unlikely for primary metabolites to be present in the rhizosphere at the millimolar levels at which they become attractive to zoospores. Do zoospores aimlessly explore the soil from where they are released until encountering this confined strip of chemoattractants? While studying the role of electro- and chemotaxis in *P. palmivora* and *Py. aphanidermatum*, van West et al. [35] proposed amino acids to not affect swimming or root targeting when at rhizosphere concentrations, but simply induce encystment close to the root surface. Unfortunately, this hypothesis is hard to validate in traditional 'swim-in' chemotaxis assays, that only monitor if a zoospore ends up at a source, and not how.

What do we know about how zoospores approach a chemoattractant source? Royle and Hickman [39,42] described the homing sequence of *Py. aphanidermatum* zoospores exposed to a source of root exudate as follows: i) disruption of typical, random helical swimming by 'milling', ii) unidirectional movement towards the attractant source, iii) trapping by frequent rapid changes of direction and iv) cessation of movement resulting in encystment. Reid et al. [70] described a similiar homing sequence for *P. palmivora*, *Py. catenulatum*, and *Py. dissotocum* zoospores toward roots, as did Allen and Newhook for *P. cinnamomi* zoospores attracted to ethanol [17]. Additionally, they reported ethanol to suppress tumbling frequencies [75]. Mitchell and Deacon [58] reported zoospores of *Py. graminicola* and *Py. aphanidermatum* to change swimming trajectories towards the host, with which they frequently collided. They also found *Py. graminicola* zoospores, after having collided with cellulose membranes, to initiate a series of escalating random changes of direction, after which they spun slowly before encysting [76]. Hardham and Gubler [77] found *P. cinnamomi* zoospores on the root surface to swim in straight lines turning abruptly, or rotate intermittently in one position. Interestingly, similar behavioural changes during attraction are also reported during electro- [34] and autotaxis [70], suggesting that swimming behaviour during positive chemotaxis is similar for other types of taxis. Studies on behavioural changes during negative chemotaxis are sparse. Royle and Hickman [39] report a zoospore-free zone around a repellent source, outside of which zoospores swim at random. Allen and Newhook [57] describe how zoospores behave in or at the border of the repellent zone; when encountering a gradient of hydrogen cation they initiate a series of turns until re-oriented away from the source.

Instead of zoospores passively aggregating through encystment induction, it thus seems that various compounds can induce active movement of zoospores towards or away from the root. Still, as initial reports on zoospore behaviour were limited to descriptions of observations, sometimes supported by figures showing zoospore trajectories by hand-drawn tracings [19,22,39] (Fig. 1F) or long exposure-imaging [39] (Fig. 1G), accounts of behaviour remained narrative and resulted in different authors ascribing different terms to what could be the same behaviour (Fig. 3). Is 'rotating' the same as 'milling', and does 'excited movement' refer to 'frequent turning'? Interestingly though, behaviour described for oomycete zoospores during their homing sequence is reminiscent of that of other microswimmers. Helical swimming is shared by virtually all protist microswimmers and allows these minute organisms to steer and orient themselves by changing the direction of the

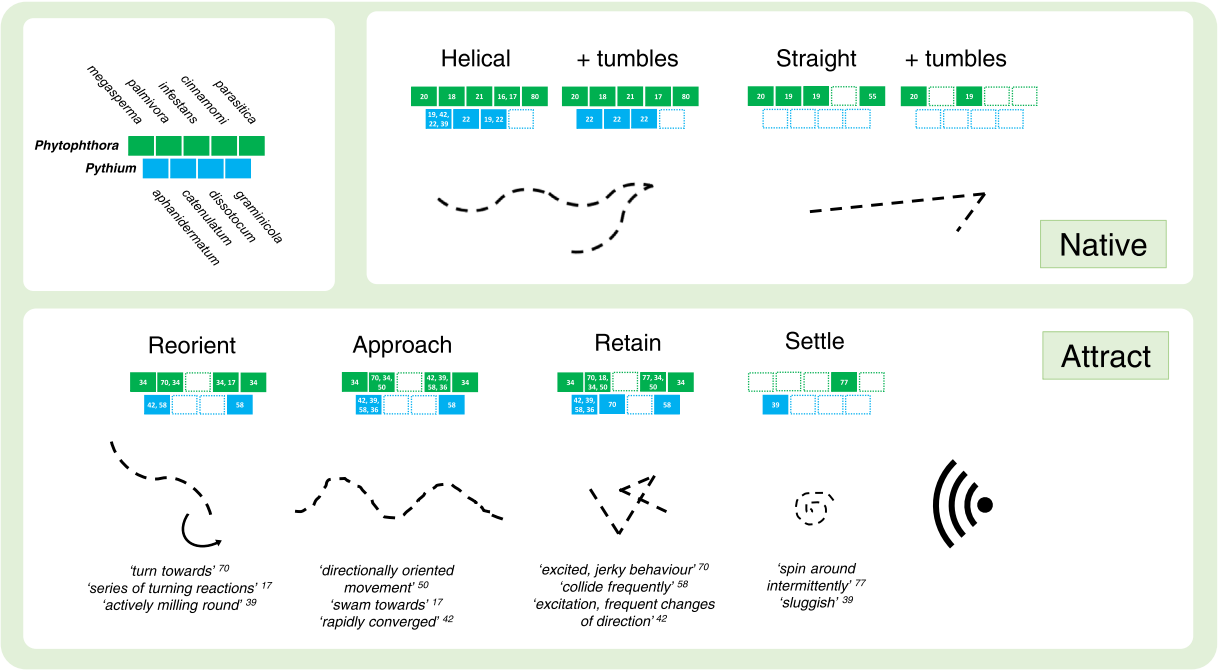

Fig. 3. Proposed terminology for describing zoospore behaviour. Top panel shows swimming patterns reported for zoospores from five *Phytophthora* species and four *Pythium* species. The numbers in the green and turquoise boxes refer to references that report zoospores to natively swim either helical or in straight stretches, and which of these references also report tumbling of zoospores. Lower panel shows reported swimming behaviour during attraction (references in boxes) with the proposed overarching terminology. The various terms used in narrative descriptions by individual authors are shown in italics.

rotational axis when encountering repellents or attractants, a behaviour known as helical klinotaxis [78,79]. Excited behaviour exhibited by oomycete zoospores when nearing an attractant resembles klinokinesis, a stochastic navigation mechanism whereby chemoattractant concentrations determine tumbling frequency, thereby biasing the movement of microswimmers up the gradient [79]. Although the most usual mechanism for klinokinesis is to decrease tumbling to migrate up a gradient, only Allen and Newhook reported the attractant ethanol to suppress tumbling [75], with an increase of turning events being reported for many other attractants. Perhaps therefore klinokinesis, although a valid strategy for chemotaxis, in oomycetes has mainly been linked to retention rather than aggregation.

In essence, the knowledge we currently have on zoospore homing is qualitative rather than quantitative. The resulting disparate terminology complicates properly aligning oomycete zoospore behaviour to that of other microswimmers, leaving the roles of reported behaviours open to interpretation. To arrive at a common language in the scientific literature we propose the following terms for the successive stages of the zoospore's homing response: reorientation, approaching, retention and settling (Fig. 3). Yet, firm establishment of a unified terminology for zoospore behaviour stands to benefit from a more precise quantification of swimming patterns. A recent study has demonstrated that such quantification is feasible. Using high-speed microscopy and automatic tracing of zoospore trajectories, Tran et al. [80] were able to directly visualize zoospore trajectories as well as flagellar beating. Their analyses revealed tumbles to occur through flagellar desynchronization: while the posterior flagellum is deactivated the anterior flagellum switches its beating pattern from sinusoidal waves to 'power and recovery strokes', and this halts and pivots the zoospore to another direction. The zoospore resumes swimming in the new direction upon reactivation of the posterior flagellum and restoration of the original anterior flagellum beating pattern. These advanced imaging techniques combined with the computational capacity to analyse superior numbers of zoospores pave the way for precise quantification of zoospore behaviour (Fig. 1H).

10. (Ab)using chemotaxis to reduce disease pressure

Already in 1961, Zentmyer, being one of the first to identify root exudates as a major cue for zoospore chemotaxis, stated: '*If it can be determined what type of chemical is attracting the spores to the root, this will provide a good basis for controlling the disease.*' [59]. In the next 60 years, Zentmyer and many other researchers demonstrated that such chemoattractants can be identified. Perhaps hoping for a 'super-attractant', they actually found a myriad of root exudate components to attract zoospores, and, even worse, the attractiveness of these components is apparently dictated by other exudate components coming along. Although research has convincingly demonstrated that root exudates play a decisive role in host specificity by attracting zoospores of compatible oomycete species, this has as yet not led to what Zentmyer [59] envisioned as 'zoospore traps', where potent attractants lure zoospores away from the plant. It seems however that abolishing attractiveness might work just as well.

One approach to abolish zoospore attraction to roots is to alter the host exudate composition. For example, deregulation of the isoflavone biosynthesis pathway in soybean by gene silencing increased susceptibility to *P. sojae*, although it was not confirmed that this was due to decreased attraction of zoospores to the root [81,82]. Alternatively, the microbiome has shown capacity to alter host root exudates to reduce attraction. Lioussanne et. al [83] found tomato root colonization by the mycorrhizal fungus *Glomus intradices* to modify root exudates in a manner that repelled *P. nicotianae* zoospores, thereby reducing disease pressure. Intercropping with plants superiorly attracting or repelling zoospores can also reduce disease pressure: Yang et al. [84] showed terpenes from fennel root exudates to not only attract *P. capsici* zoospores, but also inhibit germination and mycelial growth and reported a fennel-pepper intercrop to experience reduced disease pressure. Zhang et al. [85] reported a similar process for a maize-soybean intercrop, where maize root exudates, containing phenolic acids, repelled *P. sojae* zoospores and inhibited soybean root and stem rot.

Another approach to prevent zoospores from reaching their host is by applying strong repellents, of which the host microbiome has proven to

be a potent source. Among the many studies investigating the potential of bacteria for biological control of oomycete plant pathogens there are some that have analysed effects of bacteria or bacterial compounds on zoospore activity or behaviour. For example, massetolide A produced by the biocontrol strain *Pseudomonas fluorescens* SS101 is a cyclic lipopeptide that lyses *P. infestans* zoospores. van de Mortel et al. [86] found that at lower concentrations massetolide A induces early encystment and represses zoospore aggregation pointing to repelling activity. Shang et al. [87] identified zittermicin A and kanosamine in exudates from a *Bacillus cereus* strain and showed that these antibiotics reduce zoospore activity, encystment and germination of *Pythium torulosum* and decrease damping-off seedling mortality on tobacco roots. Mondol et al. [88] found macrocyclic trichothecenes from *Myrothecium roridum* to lyse zoospores of *P. nicotianae* and at lower concentrations to inhibit their motility. Heungens and Parke [89] even found *Burkholderia cepacia* to be able to 'mask' seed exudates, eliminating the exudate's attractiveness for *Py. aphanidermatum* zoospores. Unfortunately it is quite challenging to investigate the role of repellents or chemoattractants on zoospore behaviour in a natural setting. Metagenome analyses have revealed an enormous diversity of microbes in the rhizo- [90] and phyllosphere [91]. By now it is well established that the plant microbiome as a whole, rather than one or a few individual species, influences the fate of pathogens and determines how the plant deals with pathogen attack [92]. As yet, there are no studies addressing the issue of how effective zoospore repellents are in the natural setting.

11. A quest for receptors

The list of cues and compounds affecting zoospore aggregation and behaviour continues to grow, but up to now we have no idea how zoospores perceive and process these cues. Identifying the mechanisms of zoospore taxis would however open new avenues for disease control, for example by blocking chemoreceptors or targeting down-stream signalling pathways. So how to identify these receptors? As we know the attractants, one approach is to use these as baits to fish for their preys. A promising method that was successfully applied to directly identify receptors associated with small molecule ligands, is thermal proteome profiling that makes use of mass-spectrometry-based proteomics for selecting proteins in cell or cell lysates that change in thermal stability in the presence of a ligand [93]. An alternative approach is mining oomycete genomes for genes encoding subunits of ion channels or proteins resembling chemoreceptors identified in other organisms. Genistein and daidzein were shown to trigger calcium influx in *P. sojae*, likely mediated by membrane associated calcium channels or *myo*-inositol 1,4, 5 trisphosphate (IP_3) receptors [94,95]. An inventory of calcium channel families in oomycetes revealed several candidates, including two with a unique domain composition awaiting further characterization [96].

Likely candidates for oomycete chemoreceptors are G-protein-coupled receptors (GPCRs), a large family of cell-surface receptors in eukaryotes that are activated by a variety of ligands such as neurotransmitters and hormones, and involved in chemotaxis in many organisms [97]. This is supported by observations that zoospores of *Phytophthora* transformants lacking the Gα subunit of the heterotrimeric G-protein, a complex activated by GPCRs upon ligand binding and mediator of G-protein signalling, show aberrant swimming patterns [21] and loss of chemotaxis towards glutamic acid [21] or daidzein [98]. Apart from dozens of canonical GPCRs consisting solely of a GPCR domain, oomycetes possess several unique GPCRs in which the GPCR domain is flanked by a C-terminal accessory domain. These so-called GPCR-bigrams carry different types of accessory domains, all of which function in cell signalling and predicted to be catalytically active, hinting at oomycete-specific signalling pathways [99]. A more in depth study of *PsGK4,* the gene encoding one of 12 GPCR-PIPKs in *P. sojae* with a phosphatidylinositol phosphate kinase (PIPK) as accessory domain, showed that silencing of *PsGK4* leads to more rapid encystment and loss of zoospore chemotaxis towards daidzein and soybean roots pointing to a role for GK4 in sensing daidzein [100]. GPCRs are the targets of many pharmaceutical drugs and as such it is worth to investigate the potential of the unique oomycete GPCR-bigrams as target for oomicides. Other candidate chemoreceptors are leucine-rich repeat receptor-like kinases (RLKs). Knock-out mutants of five out of 24 RLKs identified in *P. sojae,* showed defects in chemotaxis to daidzein [101]. Like GPCRs, RLKs are cell-surface receptors that mediate signal perception and initiate downstream signalling via phosphorylation often culminating in regulation of gene expression. In this context it is noteworthy that Blanco et. al [102] identified a bZIP transcription factor in *P. infestans* that physically interacts with a zoosporogenesis-specific kinase and is required for normal zoospore movement behaviour. Further research will have to reveal whether and how GPCRs and RLKs regulate zoospore chemotaxis and what other processes are involved.

12. Outlook

Zoospores expertly sense many different chemical signals that are released in the environment by plants, other microorganisms, and even by themselves, allowing them to reach a certain target, or to avoid it. At the same time their sensory capacity and motility make zoospores vulnerable: as they are key players in the infection cycle, intentionally undermining their activity could be an effective strategy to protect plants against oomycete pathogens. One option is to exploit the array of attractants and repellents, that have already been identified. Mixing exudate components to their maximal repelling or attracting potential could allow for ecologically harmless lure and repel strategies that can be implemented in soil management practices. This can be taken further by searching for additional attractants and repellents. Plant, microbiome and zoospore exudates are far from fully explored, a striking example being the phyllosphere: zoospores tend to aggregate at stomates, but we have no clue of the taxes at play in this environment. Another option is disturbing recognition of attractants by blocking receptors. This requires an investment in identifying the essential chemoreceptors, an endeavour that benefits from the rapid increase in -omics data and advanced toolboxes for gene editing in oomycetes [103]. Another leap forward is high-speed, high-resolution microscopy that enables direct tracing of zoospore behaviour, allowing research to move from aggregation assays to navigation studies, and to truly test the mechanisms zoospores use to end up at their host. Is a single zoospore, randomly encountering a suitable host, able to attract zoospores over great distances through undiscovered potent zoospore exudates? Or do plants release high-affinity attractants, such as daidzein, that -perhaps in synergy with other components- increase the root's attractive reach beyond what is theoretically possible? In order to uncover the role of chemoattractants in the homing sequence, and thus the possibility to interfere with this homing sequence, the next step will be to identify exudate attractiveness at physiologically relevant levels in geometrically relevant environments. In turn, this implies the need for closer understanding of not simply if zoospores reach an attractant source, but more so how they accomplish this. What swimming behaviours does phytosphere geometry allow for, and does this influence the zoospore's capacity to display taxes? Definitively, direct visualization and mass quantification of zoospore behaviour as demonstrated recently [80] shows how after a 20 year silence on zoospore behavioural research we can address old questions with renewed capacity.

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Declarations of interest

none.

Data availability

No data was used for the research described in the article.

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