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Video article

Fungi, fungicide discovery and global food security

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ABSTRACT

Securing sufficient food for a growing world population is of paramount importance for social stability and the well-being of mankind. Recently, it has become evident that fungal pathogens pose the greatest biotic challenge to our calorie crops. Moreover, the loss of commodity crops to fungal disease destabilises the economies of developing nations, thereby increasing the dimension of the threat. Our best weapon to control these pathogens is fungicides, but increasing resistance puts us in an arms race against them. New anti-fungal compounds need to be discovered, such as mono-alky lipophilic cations (MALCs) described herein. Collaborations between academia and industry are imperative to establish new and efficient ways to develop these new fungicides and to bring them to the market-place.

1. The fungal challenge to our crops

Fungal diseases on crops have been increasing in severity and scale since the mid 20th Century and now pose a serious threat to global food security (overview in Fisher et al., 2012, Savary et al., 2019). Today, we lose an estimated 10–23% of our crops pre-harvest, despite disease interventions, and a further 10–20% post-harvest (Fisher et al., 2012). Fungi destroy both our essential calorie crops, such as rice, wheat, maize and soybean and decimate our commodity crops, such as bananas, coffee and barley. Indeed, the economic stability of several nations has become reliant upon export revenues, generated by the global trading of these commodity or cash crops, to buy and import food grown elsewhere in the world (Fones et al., 2020).

Modern agricultural intensification has heightened the challenge of fungal disease. Here, the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and partially protected from disease by the use of single target site antifungals, has hastened emergence of new virulent and fungicide-resistant strains (Fisher et al., 2018). Such monoculture cropping practices have, quite literally, become ideal feeding and breeding grounds for the fast emergence of new fungal variants. Indeed, their rapid life-cycles generate prolific numbers of spores and their plastic genomes, prone to mutations as well as gene acquisitions or hybridisations, generate considerable strain diversity. These pathogens are realising their true evolutionary potential, thanks to the hand of man. The race has become skewed – it is no longer between the plant and the pathogen but between the pathogen and man (Fones et al., 2020).

Climate change compounds the saga, as we see altered disease demographics as the pathogens move pole-wards in a warming world (Bebber et al., 2013). Finally, trade and transport of plants and plant products have further disseminated plant pathogens onto new hosts in hitherto unaffected areas of the world (Fones et al., 2020). For example, the livelihoods of tens of thousands who dwell in Asia and South America depend upon the commodity crop of bananas. Indeed, bananas are the most consumed, cheapest and most widely-traded global fruit (Ploetz, 2015). The recent spread of the deadly banana pathogen *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (recently renamed *Fusarium odoratissimum*, Maryani et al., 2019) to South America in 2019 jeopardises their very prosperity (García-Bastidas et al., 2020, Video 1).

Modern intensive agriculture has become the main-stay of global food security: It generates the calories needed to feed the increasing global population. However, we face a changing world blighted by known fungal adversaries, by new variants of old foes and by new crop diseases. We need better disease surveillance, more accurate predictive forecasting and new disease interventions to protect our future harvests.

2. Fungicides and the way forwards

Sustainability in the food chain must be generated by increasing disease durability. Current disease control strategies in the field include the planting of crops carrying in-bred disease resistance genes (Finckh, 2008) and the wide-spread spraying of antifungals (Oliver and Hewitt,

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2014). There is also considerable interest in the introduction of genetically modified disease resistant cultivars (Finckh, 2008), RNAi-based control strategies (Wang et al., 2016) and also of the use of microbial biological control agents (MBCAs) to protect crops against plant pathogens (Koehl et al., 2011; Kohl et al., 2019). MBCAs control disease by acting as living antagonists of plant pathogens, by producing antimicrobial compounds which suppress disease or by circumventing the pathogen to boost plant defence mechanisms (Kohl et al., 2019). However, fungicides currently remain our most potent weapon against crop pathogens. The value of fungicide is illustrated by an assessment of the economic costs of controlling Septoria tritici blotch (STB) in wheat, caused by *Zymoseptoria tritici*, in the UK. It was estimated that 20% yield losses to STB would occur in the absence of fungicide applications. These losses drop to 5–10% when control strategies are applied (Fones and Gurr, 2015), but fungicide treatments comes at a cost. However, the dividend from spraying equates to yield increases of 2.5 tons of wheat per hectare (Torriani et al., 2015). Our heavy reliance on fungicides is well-illustrated by the fact that these compounds command a worldwide value of ~13.4 billion USD (year 2019), with a projected growth of ~4.7% *per annum* over the next 7 years (https://www.alliedmarketresearch.com/fungicides-market).

Fungicides target various processes in pathogens, which fall into several categories (see the Fungicide Resistance Action Committee (FRAC) poster 2020 at https://www.frac.info/). Amongst the best understood modes of action are effects on the integrity of the plasma membrane, the microtubule cytoskeleton and the inhibition of mitochondrial respiration (Oliver and Hewitt, 2014). These processes are usually targeted by fungicides that inhibit single key enzymes. The dominant group of such single target site fungicides are the azoles, which inhibit ergosterol biosynthesis, thereby affecting the plasma and organelle membranes (Oliver and Hewitt, 2014). On the other hand, strobilurins and succinate dehydrogenase inhibitors (SDHIs) interfere with electron transfer chain in mitochondrial respiration. Together with the azoles, these fungicides account for ~77% of the market-place (Oliver and Hewitt, 2014). However, while single-target site fungicides are specific and effective in controlling fungal pathogens, they come with a major risk of resistance development. Indeed, point mutations in the active centre of their target enzymes can result in strong resistance within a few years of field usage (Oliver and Hewitt, 2014). This challenges not only crop protection strategies, it also poses a threat to clinical applications, as we see cross-over of resistance from crops to the clinical setting, evidenced by the emergence of drug-resistant strains of *Aspergillus fumigatus* (Fisher et al., 2018). In order to keep ahead of this arms race with fungi, we must constantly develop new antifungal compounds. Ideally, such novel fungicides should fulfil several criteria: They should be (i) effective against a broad range of important crop pathogens, (ii) affect essential processes in the fungal pathogens in multiple ways, thereby reducing the risk of resistance development; (iii) of low toxicity to non-target organisms, including humans, animals and plants; (iv) activate the plant defence system, thereby priming the plant for a potential pathogen attack (Hewitt, 2000; Leadbeater, 2015).

So far, fungicide development has been largely orchestrated within the agrochemical crop protection industries (Russell, 2006; Leadbeater, 2015; Sparks and Lorsbach, 2017). Here, the dominant strategy appears to use lead compounds to develop more efficient variations of existing fungicide molecules. A good example of such a product is the fungicide Revysol®, an azole fungicide most recently introduced to the market (https://agriculture.basf.com/global/en/innovations-for-agriculture/innovation-for-fungicides/revysol.html). Academic research, on the other hand, largely focusses on aspects of antifungal mode of action or resistance emergence in pathogen populations (e.g. Hewitt, 2000; Hobbelen et al., 2014; Lucas et al., 2015). However, a recent study reports a more active role in fungicide discovery. Here, the combinatorial use of fungal cell biology, plant pathology, synthetic organic chemistry and toxicology led to the discovery of a new compound, which holds the potential to become a multi-site fungicide (Steinberg et al., 2020). This study used a more targeted approach, following a sequence of questions, which are summarised below:

Question 1: Which essential cellular processes in fungal pathogens are sufficiently different from non-target organisms (animals, plants) to develop a fungal-specific inhibitor? Using published literature, the authors identified fungal mitochondrial respiration as such a target, being characterised by unique and fungal-specific respiratory proteins (Affourtit et al., 2000; Joseph-Horne et al., 2001). Targeting this process is attractive, as it may obviate negative effects on non-target organisms, such as humans or plants.

Question 2: How could one target this process? A review of published literature revealed that lipophilic cations are used to deliver therapeutics to human mitochondria (Zielonka et al., 2017). These molecules accumulate in the matrix of mitochondria, from where they insert into the inner mitochondrial membrane (Murphy, 2008; Zielonka et al., 2017), and thus may target the respiration chain.

Question 3: Are any lipophilic cations used as fungicides? The authors identified the protective fungicide dodine, which consists of a cationic head group, fused to a lipophilic n-alkyl chain. Dodine is used in the field

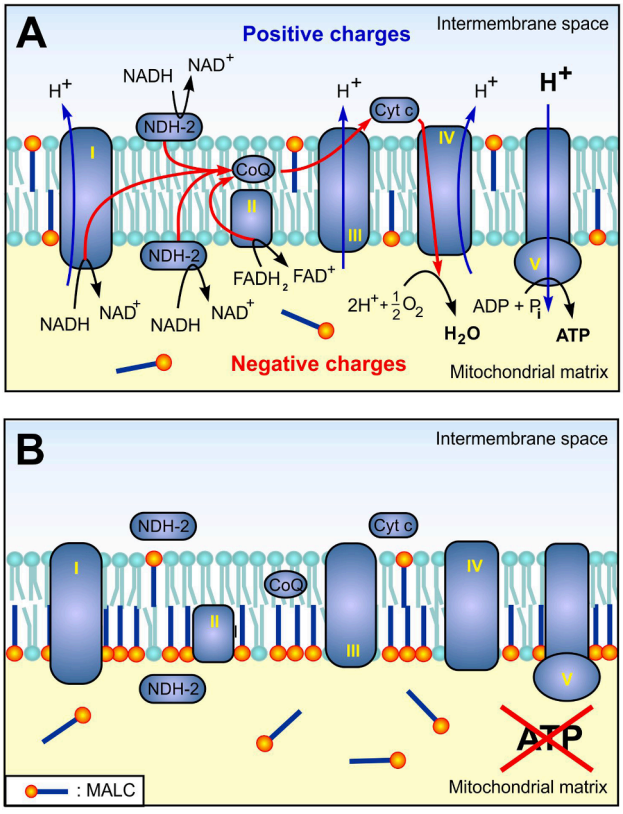
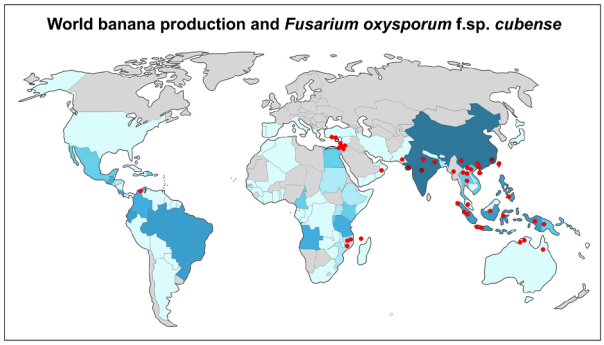


Fig. 1. Effect of MALCs on the fungal respiration chain. MALCs consist of a lipophilic n-alkyl chain and a cationic head group. They have an overall lipophilicity, given as the LogP value, that allows passage through cellular membranes (e.g. $LogP_{Dodine} = 2.26$, $LogP_{ATP} = -3.39$, $LogP_{Membrane\ phospholipid} = +10.19$; see Steinberg et al., 2020). Due to their cationic head group, MALCs accumulate in mitochondria, the only negatively-charged organelle in the cell (**A**). Here, they insert into the inner mitochondrial membrane, which holds the enzymes for cellular respiration and ATP-synthesis. MALCs inhibit NADH oxidation, which involves unique and fungal specific respiratory enzymes (**A**, NDH-2 = alternative NADH dehydrogenases, Joseph-Horne et al., 2001). The inhibition of mitochondrial respiration reduces cellular ATP levels and, ultimately, kills the pathogen (**B**). A newly-synthesised MALC (C_{18}.SMe_2^+) induces the formation of reactive oxygen species (ROS) at respiratory complex I. This, in turn, activates apoptosis and drives the pathogen to commit "cell suicide" (apoptotic cell death). These multiple modes of action (Video 1) underpin effective protection by C_{18}.SMe_2^+ against Septoria tritici blotch in wheat and rice blast disease. The Figure was modified from Steinberg et al. (2020).

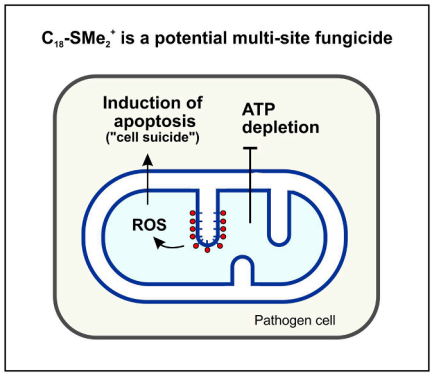


Video 1. Worldwide production of bananas and reported cases of the Panama disease-causing pathogen F. oxysporum f.sp. cubense, tropical race 4 (FocTR4). The pathogen was first reported in East Asia, from where it spread to Southeast Asia and Australia. More recently, FocTR4 moved westwards and appeared in Africa, Israel, Turkey and South America. Note that Latin America holds two-thirds of the global banana market. Banana production data are based on data provided by the UN Food and Agriculture Organization, as summarised in Ritchie, 2020 (https://ourworldindata.org/agricultural-production). The years of appearance of Tropical Race 4 are either the date of symptom recognition or the publication date. The information of Tropical Race 4, summarised in this Video1, have been collected and provided by the ProMedusa network. For further information see http://www.promusa.org/Tropical+race+4+-+TR4#Distribution.

to control scab on apples, pears and several foliar diseases of cherries, strawberries, peaches, yet its mode of action (MoA) is classified as "U12, Unknown" in the FRAC code© list 2020 (see https://www.frac.info). This is due to contradictory reports, claiming fungicidal activity of dodine either by disrupting the plasma membrane or affecting metabolism (overview in Schuster and Steinberg, 2020). To clarify the MoA, the authors used fluorescent reporter strains of *Z. tritici*, alongside live cell imaging techniques (Kilaru et al., 2017; Schuster et al., 2015). These studies demonstrate that dodine does, indeed, inhibit mitochondrial respiration, so depleting the pathogen of ATP and propelling it towards death (Fig. 1; Video 2).

Question 4: Can one design a better mono-alky lipophilic cation (MALC)? The authors used the knowledge of the MoA to test extant, and newly-designed and synthesised molecules, using dodine as the lead compound. This revealed a novel MALC, named C_{18}-SMe_2^+. This molecule not only inhibits the respiration chain and ATP synthesis, but also induces reactive oxygen species (ROS). This, in turn, activates a programmed cell death pathway. Thus, C_{18}-SMe_2^+ has a multiple MoA; it depletes the pathogen's energy supply and drives the cell to commit "suicide" (apoptosis: Video 2). Moreover C_{18}-SMe_2^+ activates the plant defence system prior to fungal attack. The study also shows effective protection by C_{18}-SMe_2^+ against Septoria tritici blotch and rice blast disease, respectively (Steinberg et al., 2020), which is most likely due to these multiple activities.

Question 5: Is the new MALC environmentally benign and of low toxicity to non-target organisms? Fungicides can be toxic to non-target organisms (e.g. as reported with the widely-used fungicide Chlorothalonil being toxic to aquatic organisms [Van Scoy and Tjeerdema, 2014] or the pollution of soil by heavy metal-based fungicides (e.g. copper contamination of soil [Loland and Singh, 2004)]). As a first step towards an understanding of the toxicity of C_{18}-SMe_2^+, the authors performed several tests. They found no phytotoxicity in rice or wheat, no genotoxicity in AMES tests and only low toxicity in human culture cells and in water fleas (*Daphnia magna*). While these assays are most encouraging, registration of a new fungicide requires further defined toxicological testing and environmental fate investigations (McGrath, 2016).



Video 2. Summary of the MoA of mono-alkyl lipophilic cations (MALCs), including that of the novel compound C18-SMe2+ (Steinberg et al., 2020).

3. Conclusions

Fungi pose the most serious biotic threat to food security. Fungicides remain our most potent and immediate answer to this threat. However, increasing resistance development makes the discovery and development of new fungicides of paramount importance. Here, we describe a recently published targeted approach to develop a new anti-fungal compound. Using the MALC fungicide dodine as a lead chemistry, a more potent chemistry was identified. However, it is a long and costly route from the laboratory to the product. From formulation, through development and registration to field application can take over 11 years and cost in excess of ~250 million Euros (https://croplife.org/wp-content/uploads/2018/11/Phillips-McDougall-Evolution-of-the-Crop-Protection-Industry-since-1960-FINAL.pdf). Academic research can support the quest to discover new anti-fungal compounds. Close collaboration between academia and industry will speed up discovery of alternative and more resilient crop protection compounds, needed to secure our food in future times.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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