

## RESEARCH ARTICLE

# Profile of the *in silico* secretome of the palm dieback pathogen, *Fusarium oxysporum* f. sp. *albedinis*, a fungus that puts natural oases at risk

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## Abstract

Understanding biotic changes that occur alongside climate change constitute a research priority of global significance. Here, we address a plant pathogen that poses a serious threat to life on natural oases, where climate change is already taking a toll and severely impacting human subsistence. *Fusarium oxysporum* f. sp. *albedinis* is a pathogen that causes dieback disease on date palms, a tree that provides several critical ecosystem services in natural oases; and consequently, of major importance in this vulnerable habitat. Here, we assess the current state of global pathogen spread, we annotate the genome of a sequenced pathogen strain isolated from the native range and we analyse its *in silico* secretome. The palm dieback pathogen secretes a large arsenal of effector candidates including a variety of toxins, a distinguished profile of secreted in xylem proteins (SIX) as well as an expanded protein family with an N-terminal conserved motif [SG]PC[KR]P that could be involved in interactions with host membranes. Using agrobiodiversity as a strategy to decrease pathogen infectivity, while providing short term resilient solutions, seems to be widely overcome by the pathogen. Hence, the urgent need for future mechanistic research on the palm dieback disease and a better understanding of pathogen genetic diversity.

## Main

Global climate has changed rapidly over recent decades, and climate-change predictions in some water-limited regions, such as westernmost Mediterranean (Iberia and Morocco), North Africa and Middle East, forecast a significant shift in the near future, with less frequent precipitation and hotter and longer drought events [1, 2]. An impact of climate change has already been observed on biodiversity and crop productivity; and consequently, on human livelihoods in affected areas [3]. Because of its desert location, vulnerable ecosystem and farming practices, oases are at major risk [4].

Alongside abiotic stresses caused by global warming, natural oases are increasingly facing threats by emerging infectious plant diseases, notably those caused by fungal and oomycete pathogens (Table 1). In terms of yield losses, fungi are the most destructive emerging pathogens in oases, posing a serious threat to food security and oasis ecosystem health. While many emerging diseases in oasis ecosystems are encountered in other agroecological zones [5], a few diseases are exclusively of oasis origin. One such disease is dieback of palm, also known as Bayoud, caused by the ascomycete fungus *Fusarium oxysporum* f. sp. *albedinis*. The palm dieback pathogen has been identified in the late 19<sup>th</sup> century in the oasis of Zagora, Southern Morocco [6], but it has only started to spread rapidly in recent decades. Palm dieback has now been detected in many palm-growing areas across Africa, Asia and America [7–11], with North Africa being the most affected. An estimated 15 million palm trees in oasis habitats of Morocco and Algeria have now been completely wiped out by the palm dieback disease, which continues to progress at an alarming rate despite prophylactic measures to contain pathogen spread [11]. When outbreaks of palm dieback take a pandemic scale, natural habitats collapse entirely (Fig 1), severely impacting human livelihoods, economy and subsistence in populated oases.

Date palm (*Phoenix dactylifera*) is a distinct species that belong to the palm family (Arecaaceae of the monocotyledon order Arecales) [12]. Date palms are pivotal components of natural oasis ecosystems, where they provide many ecosystem services, such as protection from silting and desertification, cooling oasis temperature, creating a suitable microclimate that supports the development of other crops, changing soil-water dynamics, and supporting wildlife as well as providing food and animal feed. Through these diversified ecosystem services, date palm contributes to the long-term functioning and, notably, the resilience of oasis ecosystems to natural and anthropogenic perturbations [13]. Most date palm cultivars are susceptible to the dieback disease and have already succumbed in North Africa despite efforts of restoration [7, 10]. Palm dieback disease is jeopardising the genetic diversity of date palm in affected oases, especially when spreading at an epidemiological rate, wiping out groves and, sometimes, entire oases (Fig 1A). Besides, the number of palm trees lost to dieback disease may be underestimated, as farmers stop irrigating entire groves and regions of the oases where the disease has erupted as an effort to limit pathogen spread, thereby killing not only domesticated but also date palm's wild relatives that represent a reservoir of genetic diversity, which is needed for agricultural improvement.

**Table 1. Emerging infectious plant diseases in natural oases.**

Species	Disease	Pathogen	Reference
<i>Phoenix dactylifera</i>	Dieback (Bayoud) disease	<i>Fusarium oxysporum</i> f.sp. <i>Albedinis</i>	(Toutain, 1965; Louvet and Toutain 1973; Sedra, 1992)
<i>Phoenix dactylifera</i>	Brittle leaf disease	Unknown	(Namsi et al., 2007)
<i>Phoenix dactylifera</i>	Lethal yellowing disease	<i>Candidatus Phytoplasma palmae</i>	(Harrison et al., 2008)
<i>Phoenix dactylifera</i>	Inflorescence rot disease	<i>Mauginiella scaettae</i>	(Taxana and Larous, 2003)
<i>Phoenix dactylifera</i>	Diplodia leaf-base disease	<i>Diplodia phoenicum</i>	(El-Deeb et al., 2007)
<i>Phoenix dactylifera</i>	Belaat disease	<i>Phytophthora palmivora</i>	(Sedra, 2003)
<i>Phoenix dactylifera</i>	Black scorch disease	<i>Thielaviopsis punctulata</i>	(Al-Naemi et al., 2014)
<i>Phoenix dactylifera</i>	Bending Head Disease	<i>Botryodiplodia theobromae</i>	(Sedra, 2003)
<i>Citrus aurantifolia</i>	Witches' Broom disease	<i>Candidatus phytoplasma aurantifolia</i>	(Khan & Grosser, 2004)
<i>Olea europaea</i> spp.	Root rot disease	<i>Cylindrocarpon destructans</i> , <i>Phytophthora</i> spp.	(Zizzerini and Marte, 1976; SIPAM, 2008)
<i>Malus pumila</i>	Fire Blight disease	<i>Erwinia amylovora</i>	(Fatmi et al., 2008; SIPAM, 2008)
<i>Solanum lycopersicum</i>	Tomato wilt	<i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i>	(SIPAM, 2008; Murugan et al., 2020)
<i>Solanum melongena</i>	Root-knot disease	<i>Meloidogyne arenaria</i>	(SIPAM, 2008; Mokrini et al., 2019)
<i>Vitis vinifera</i>	Pierce's disease	<i>Xylella fastidiosa</i>	(Choi et al., 2013)

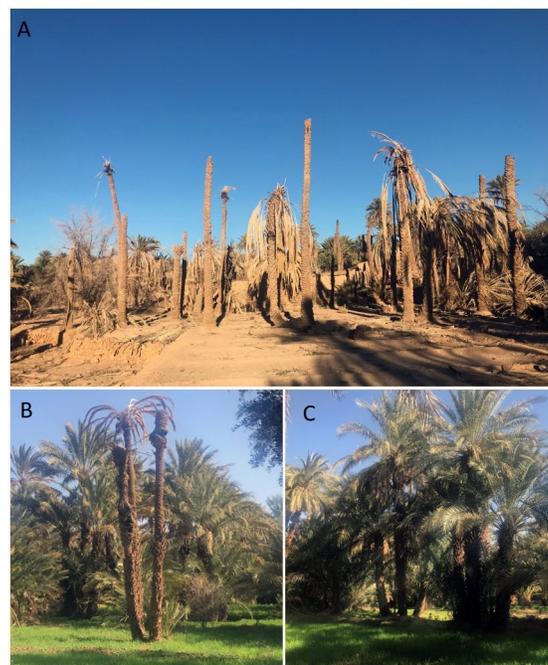
<https://doi.org/10.1371/journal.pone.0260830.t001>

Palm dieback disease is characterised by discolouration from green to whitish of one palm leaf of the middle crown, followed by necrosis on the dorsal side of the rachis that progresses from the base to the tip of the frond, corresponding to mycelia progression in the vascular bundles of the rachis. After one leaf has been infected, adjacent leaves show the same succession of symptoms. This leads to crown dieback and ultimately the death of infected trees [14] (Fig 1B). All commercialised date palm cultivars seem to be equally susceptible to the disease, which affects young offshoots as well as over 200-year-old palms. Albeit alarmingly destructive, pathogenesis of the disease is as yet uninvestigated and molecular details of how *F. oxysporum* f. sp. *albedinis* brings about infection of palm trees are still unknown. Secretome profiling and predictive ranking of effector candidates of this pathogen have, so far, not been addressed in research. It is therefore timely to address the palm dieback pathosystem in order to understand how the disease takes place and, subsequently develop disease control strategies that can save date palm trees and natural oases. Genome sequence data of only one strain (Foa 133) of *F. oxysporum* f. sp. *albedinis* has been made available so far [15]. Here, we annotate the draft genome of Foa 133 strain, we mine its secretome, we analyse its large repertoire of effector candidates, and we briefly discuss research areas that should be the focus of future studies in order to get insights into Foa's molecular mechanisms of pathogenicity.

## Results and discussion

### Genome annotation and secretome profiling of *F. oxysporum* f. sp. *albedinis*

To mine the secretome of the palm dieback-causing agent *F. oxysporum* f. sp. *albedinis* (Foa), we annotated the draft genome of the only isolate sequenced so far Foa 133 strain [15], as described in the methods section. The draft genome annotation data is hosted in the public



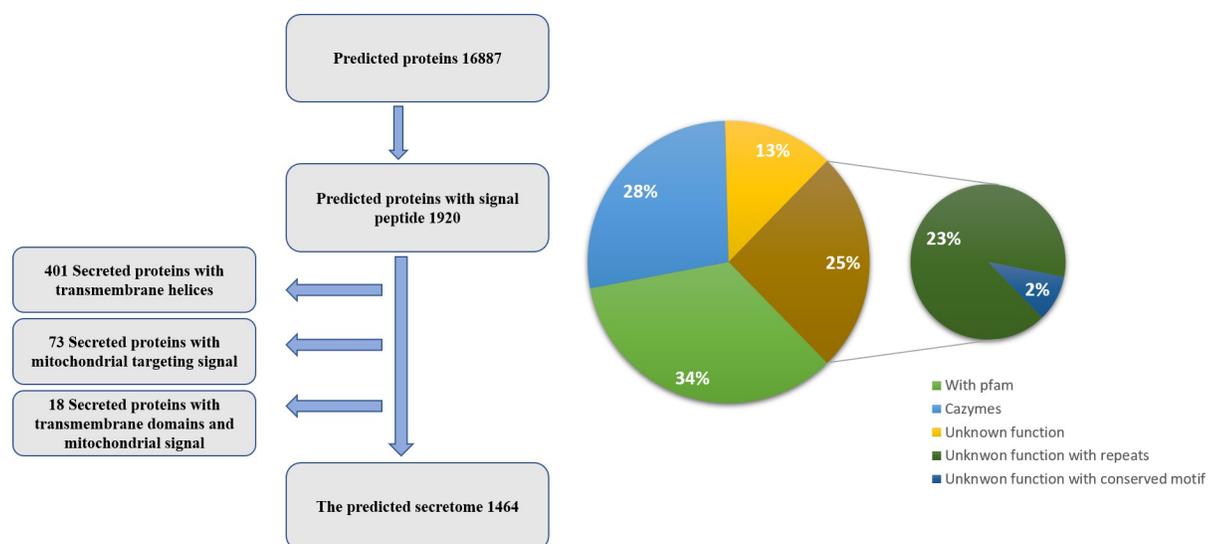
**Fig 1. Dieback of date palm in a natural oasis in Morocco.** (a) Palm dieback disease taking a pandemic scale and leading to ecological collapse of a Moroccan oasis. (b) Date palm tree showing dieback on the crown. (c) Healthy date palm.

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repository Zenodo (DOI: [10.5281/zenodo.5815342](https://doi.org/10.5281/zenodo.5815342)) (<https://zenodo.org/record/5815342#.YfPv9f7MI2w>). Annotation of the identified 16887 genes is summarised in [S1 File](#). The secretome was determined by processing predicted protein datasets through an *in-silico* secretion pipeline ([Fig 2](#)). In this study, the secretome is defined as proteins that are predicted to be secreted extracellularly through the classical ER secretory pathway, do not target mitochondria and do not integrate into the membrane through transmembrane domains (TMs). Of the predicted 16887 genes, 1464 were predicted to code for secreted proteins, of which 1077 (73%) contained less than 500 amino acids and 598 proteins had no identified pfam domain ([Table 2](#)). Annotation of all secreted proteins is presented in [S1 Table](#). Foa secretome harbours a large arsenal, 386, of carbohydrate active enzymes (CAZymes) that are predicted to be active in the apoplast, contributing mainly to plant cell wall degradation. These predicted CAZymes are distributed among glycoside hydrolases (GH), auxiliary activities (AA), carbohydrate esterases (CE), pectin lyases (PL), carbohydrate binding modules (CBM) and glycosyl transferases (GT) families, harbouring 209, 107, 26, 37, 9 and 3 members, respectively. 73 GH and CE proteins are accompanied by CBM modules ([S2 Table](#)). 550 predicted secreted proteins contain cysteines that are predicted to form bisulfide bridges. Of these, 437 proteins contain at least two predicted disulphide bonds ([S3 Table](#)). 313 secreted proteins were predicted to carry a wide range of enzymatic functions. 370 proteins (25%) contain repeats, carrying two or more copies of a tandemly or non-tandemly duplicated sequence or structural motif that is at least five amino acid residues in length. We have not considered degenerate sequence repeats that may be identifiable only through analysis of protein tertiary structure. Proteins carrying LysM domains were also recovered in the secretome. One protein (FUN\_010192-T1) carried *Alternaria alternata* allergen 1 pfam domain PF16541.4.

### A diversified group of necrosis-inducing proteins among candidate effectors of *F. oxysporum* f. sp. *albedinis*

Within fungal secretomes, effectors have been widely investigated for their role in fungal virulence and disease development in plants [16–18]. Fungal effector proteins are predicted to function either in the interface between pathogen and host cell structures, or inside host cells,



**Fig 2. *F. oxysporum* f. sp. *albedinis* genome code for a large repertoire of secreted proteins.** A. Overview of the computational pipeline used to mine predicted secreted proteins. B. Pie chart showing percentages of the main protein groups in the secretome.

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**Table 2. Composition of the predicted secretome of *F. oxysporum* f. sp. *Albedinis*.**

Class	Number of proteins
Secreted	1464
Less than 500 amino acids	1077
No identified pfam domain	598
Cysteine rich	550
CAZymes	386
Repeat containing	370

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where they manipulate molecular processes in the host in favour of disease development [19]. When they are intercepted by the host immune system, effectors of biotrophic pathogens are called avirulence proteins because their recognition by the host surveillance system causes localised plant cell death (PCD) that limits pathogen virulence and spread [16, 18, 20–22]. In some necrotrophic pathosystems, however, effectors triggering PCD function as positive regulators that enhance further disease development because they make nutrients available to the pathogen from the resulting dead plant tissue. This is referred to as necrotrophic effector-triggered susceptibility (NETS) [23–27]. Typically, effectors are under diversifying selection either to circumvent the host immune surveillance or to adapt to environmental pressures and, therefore, evolve relatively fast [28], making their prediction challenging. Currently, many thousands of effector candidates have been predicted in genomes of filamentous pathogens and their expression profiles have been analysed, yet for any identified fungal pathogen only a few fungal effectors have been functionally characterised and shown to play a role in fungus-plant interactions [20, 29]. Nevertheless, certain features have been found to be shared between fungal effectors that make their prediction, albeit inaccurate, possible. Such features include harbouring a signal peptide for secretion outside fungal structures, small size, cysteine content [30–33], a higher sequence diversity than rest of genes [34] or, in rare cases, also harbouring conserved motifs [35]. To enable uncovering of potential virulence proteins deployed by *Foa* to invade date palm tissue, we have identified candidate effectors within *Foa* secretome based on cysteine content (at least 2 counts of predicted disulphide bonds) and using EffectorP 2.0 [36] (S2 File). We found that candidate effector proteins account for 30% of the *Foa* secretome. Among these, we recovered three homologues of necrosis-inducing toxins called Nep1-like proteins (NLPs), which contain the nlp24 peptide with its two conserved regions I (11-aa immunogenic part) and II (the heptapeptide GHRHDWE motif) [37]. Three other candidate effectors with predicted phytotoxicity and homology to *Cladosporium fulvum* Ecp2 effector (Hce2) [38] were recovered in *Foa* secretome (Table 3).

NLPs were first identified in *Fusarium oxysporum* f. sp. *erythroxyli* culture filtrate and were initially called ‘necrosis and ethylene-inducing proteins’ (NEP1) {Bailey, 1995 #256}. Necrosis-inducing secreted proteins have, subsequently, been found in a range of plant-associated

**Table 3. Potential necrosis-inducing toxins encoded in the *F. oxysporum* f. sp. *albedinis* genome.**

Protein ID	pfam accession	pfam names	pfam evalues
FUN_007374-T1	PF05630.10	Necrosis inducing protein (NPP1)	1.70E-53
FUN_001358-T1	PF05630.10	Necrosis inducing protein (NPP1)	1.10E-56
FUN_000270-T1	PF05630.10	Necrosis inducing protein (NPP1)	8.30E-65
FUN_004711-T1	PF14856.5	Pathogen effector putative necrosis-inducing factor (Hce2)	1.50E-19
FUN_009556-T1	PF14856.5	Pathogen effector putative necrosis-inducing factor (Hce2)	8.00E-30
FUN_007640-T1	PF14856.5	Pathogen effector putative necrosis-inducing factor (Hce2)	5.9E-7

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microorganisms, including oomycetes, fungi and bacteria, and tend to cause necrosis mostly in dicotyledonous plants [39, 40]. Disruption of NLP genes in *F. oxysporum* f. sp. *erythroxyli* and some other pathogens does not affect virulence [Bailey, 2002 #257][Cuesta Arenas, 2010 #259]. Instead, NLPs are suggested to function as virulence factors that accelerate disease development and pathogen growth in the host [Amsellem, 2002 #266][Feng, 2014 #270]. It is unclear whether NLPs identified in Foa genome are causing necrosis observed in infected monocotyledonous date palm rachises. In fungi, ascomycetes are known to secrete NLPs that contain six conserved cysteine residues. Interestingly, one of Foa's predicted NLPs possesses 2 cysteine residues (Table 3), making necrosis-inducing secreted proteins intriguing candidates for experiments investigating their role in virulence.

Other secreted proteins with putative cytotoxic functions include candidate effectors of predicted S1/P1 Nuclease (FUN\_003983-T1) and Ribonucleases T2 (FUN\_001364-T1 and FUN\_008322-T1) families, that might either act intracellularly as a cytotoxin by scavenging host nucleic acids [41, 42] or extracellularly to inactivate the damage-associated molecular pattern extracellular DNA [43, 44]. S1/P1 nucleases are abundant in the secretomes of fungal pathogens, including the necrotrophic pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea* [45], the hemibiotrophic pathogen *Hymenoscyphus fraxineus* [34] and the obligate biotrophic pathogen *Puccinia triticina* [46]. However, only a few examples of this group of phytotoxic effectors have, so far, been functionally studied. These include the ribonuclease-like effector CSEP0064/BEC1054 of *Blumeria graminis*, which interferes with the degradation of the host ribosomal RNA. The ribonuclease activity of CSEP0064/BEC1054 effector is thought to suppress plant immunity by inhibiting the action of plant ribosome-inactivating proteins (RIPs) and subsequently suppressing PCD that would otherwise limit viability of the biotrophic pathogen on the host [47]. Similarly, fungal DNase has been shown to play a role in the pathogenesis of the maize fungal pathogen *Cochliobolus heterostrophus*, perhaps as a counter defence mechanism against host-secreted extracellular DNA [44].

### ***F. oxysporum* f. sp. *albedinis* harbours a distinct profile of SIX genes**

Fourteen effectors referred to as secreted in xylem (SIX) proteins, some with a proven role in virulence, have been identified in *F. oxysporum* f. sp. *lycopersici* [48–50]. In tomato and cucumber-infecting strains, SIX genes were found to be located on small, dispensable accessory chromosomes. Horizontal transfer of these chromosomes from pathogenic lineages of *F. oxysporum* to a non-pathogenic recipient isolate of *F. oxysporum* renders the latter pathogenic on the respective host [51, 52]. Four sequences similar to SIX effectors were recovered from the draft genome of Foa 133 strain. Two sequences are variants of SIX1 and two other sequences are similar to SIX9 and SIX11 (S3 File). The last two SIX effectors (SIX 9 and SIX11) have, so far, only been found in pathogenic species of *Fusarium* sp [53]. Based on this finding, the set of SIX genes present in Foa 133 isolate presents a pathogenic profile and is distinct from previously identified SIX gene profiles in *formae speciales* of *F. oxysporum*, supporting the hypothesis that each *forma specialis* possesses a unique combination of effectors. However, as only a single Foa strain has so far been sequenced, more population genomics and functional studies will be needed to determine the profile and the function of SIX genes in *F. oxysporum* f. sp. *albedinis*.

### **[SG]PC[KR]P effector candidates**

We screened the entire predicted secretome of Foa 133 for conserved motifs that have previously been identified in predicted effectors of other filamentous plant pathogens, such as RxLR [54, 55], YxSL[RK] [56], [YFW]xC [57], [LI]XAR [58], [RK]CxxCx<sub>12</sub>H [58], G[I/F/Y][A/L/S/

T]R [59] and DELD [60]. While some of these short motifs are frequently found in many Foa secreted proteins, they do not occur at the correct position within Foa secreted proteins and may simply be due to artifacts of random background matches (motif occurrence is included in S2 Table). On the other hand, 34 putative effectors in Foa's secretome carry the conserved motif [SG]PC[KR]P immediately following the predicted signal peptide for secretion (S4 File). This motif has, so far, only been detected in *Fusarium* proteins [61]. Effector candidates with this motif seem to be rapidly evolving and have been suggested to play a role in pathogenesis [62], though functional studies have not as yet been published. [SG]PC[KR]P effector candidates have been identified in the secretomes of *F. graminearum*, *F. pseudograminearum*, *F. oxysporum* and *F. solani* secretomes [61]. In all four species, all proteins carrying this motif also contain cysteines. In Foa's secretome, [SG]PC[KR]P candidate effectors are predicted to be heavily glycosylated and phosphorylated. They also carry repeats of PAN/apple domains. The PAN/apple domain (PF00024, PF14295) is enriched in the secretomes of oomycete species and is associated with carbohydrate-binding modules, such as cellulose-binding elicitor lectins (CBEL), which also elicit strong host immune responses when infiltrated into host (tobacco) and non-host plants, including *Arabidopsis thaliana* [63–67]. On the other hand, PAN/apple modules are also involved in protein-protein interactions [68]. They are found on *Toxoplasma* cell surface binding receptors that are involved in cell entry [69, 70]. A noted feature of PAN/apple domain-containing proteins is a conserved pattern of cysteine residues. Seven of the 34 identified Foa [SG]PC[KR]P effector candidates are not predicted to carry any disulphide bond, but are still predicted to carry several CBMs, suggesting a binding activity. Based on homology detection and structure prediction by HMM-HMM comparison (HHpred), [SG]PC[KR]P effector candidates are overall predicted to have a hydrolase activity and to interact with components of the plant cell membrane. This interaction is likely to be mediated through N-terminal noncatalytic CBM domains, whose role could be to bring [SG]PC[KR]P proteins in close proximity to their substrates. In addition, HHpred prediction highlights homology signatures to several membrane-interacting and pore-forming proteins, such as bacterial adhesins, vegetative insecticidal proteins Vip3 and Vip4, viral capsid proteins, and cellulosomal scaffoldin adaptor protein B (S4 Table).

In Foa 133 genome, [SG]PC[KR]P motif seems to occur exclusively in secreted proteins. Only three non-secreted proteins, FUN\_007157-T1, FUN\_010458-T1 and FUN\_010145-T1 carry [SG]PC[KR]P motif, although not at the right position. Interestingly, one of these hypothetical proteins, FUN\_010458-T1, is also predicted to carry two PAN domains. Based on these bioinformatics predictions, we hypothesize that [SG]PC[KR]P effectors could form a machinery of proteins, such as cellulosome or equivalent, in *F. oxysporum* f. sp. *albedinis*, and perhaps also in other *Fusarium* species. A complex consisting of a variety of different enzymes bound to noncatalytic scaffolding subunits with a role of binding mediators, which can each bind, perhaps specifically hence sequence diversity, to one of the various plant cell surface anchoring proteins. [SG]PC[KR]P proteins will be part of our priority list for mechanistic and functional studies in the date palm dieback pathosystem.

## Conclusions

Palm dieback disease is killing date palms, the cornerstone of life in Saharan oases, and posing a threat to biodiversity as well as to human subsistence and livelihoods in populated oases. The disease is progressing at an alarming rate, moving west to east across northern Africa and reaching as far as the Middle East and Pakistan (S1 Fig) [11]. In an effort to protect date palm cultivars of economic importance and restore it to oases landscapes, millions of genetically diverse palm trees demonstrating different levels of tolerance to the highly destructive palm

dieback pandemic have been planted over the last two decades across Morocco's oases under agroforestry systems [71]. However, while diversifying host populations has the potential to challenge the pathogen infectivity through variation in host susceptibility, disease progress and spread of infection depends equally on the level of genetic diversity in the pathogen population [72]. As such, future research should aim at analysing pathogen strains across infected areas and address more empirical studies. In order to get insights into the mechanisms of infection deployed by *Foa* to invade palm tissue, we annotated the first draft genome of *Foa* strain 133 and we analysed its *in silico* secretome, including putative secreted effectors. Two particular sets of secreted proteins, SGPCKRP and SIX effector candidates, are specific to *Fusarium oxysporum* but have their contribution to the pathogen's virulence as yet uncharacterised. These proteins will be prime candidates for our future studies. Several other effector candidates have predicted cytoplasmic functions and are suggested to be transferred into infected plant cells. These effector candidates are likely to be required more during the initial biotrophic growth of the pathogen than upon its later necrotrophic development. One limitation of this *in silico* study is the absence of experimental data. Empirical analyses will be needed not only to confirm bioinformatics prediction of the secretome, but also to identify the whole spectrum of *Foa*'s exported proteins that play a role in fungal colonisation of date palm, including proteins that are exported via non-conventional secretion systems. Nevertheless, the pipeline used here has generated several groups of secreted proteins and potential effectors homologous to those that have been either studied *in vivo* or found in predicted secretomes of other fungal pathogens. Some of such proteins include CAZymes, LysM proteins, necrosis-inducing proteins, SIX proteins and [SG]PC[KR]P proteins. When compared to other *formae speciales* of *Fusarium oxysporum*, *Foa*'s secretome mined in this study harbours a large repertoire of predicted effector genes. This is in line with the pattern that has, recently, been discovered in pathogenic species of *F. oxysporum*, where genomes of pathogenic strains tend to code for a wider range of effector proteins whilst endophytic genomes tend to contain fewer effector genes (Constantin et al., 2021). In addition, the large size of *Foa* secretome is in accordance with previous findings that pathogenic fungi secrete larger numbers of proteins than symbionts (Kim et al., 2016). *Fusarium* species are likely to have had a long evolutionary history with North African palm trees and may associate with this host in a range of different interactions spanning the spectrum from harmless endophytism to pathogenesis. What caused the outbreak of palm dieback in recent decades is as yet unknown. The increased frequency of severe long and hot drought events in Saharan oases under an ever-changing climate may render the palm dieback pathogen harder to detect and mitigate at the onset of disease development. It is, therefore, timely to address both biochemical and genomics studies in order to elucidate palm dieback disease function and help save date palms, which in turn will help conserve oasis ecosystems.

## Materials and methods

### Annotation of *Fusarium oxysporum* f. sp. *albedinis* strain 133

We used the assembled genome of *Fusarium oxysporum* f. sp. *albedinis* strain 133 [15]. Gene prediction and annotation were carried out using funannotate pipeline v1.8.3 [73], which includes masking, ab initio gene-prediction training, using Augustus and Genemark, with the EST dataset reported to the Ganoderma mycocosm repository, gene prediction, and the assignment of functional annotation to protein-coding gene models. As input for the prediction step we used extrinsic protein evidence from the publicly available annotation (GCF\_000149955.1) of *Fusarium oxysporum* f. sp. *lycopersici* 4287 and from the UniprotKB/swiss-prot database. The annotation workflow is hosted in the public repository github ([https://github.com/lukasjelonek/evaluate\\_funannotate](https://github.com/lukasjelonek/evaluate_funannotate)).

## Secretome prediction

We used a pipeline described previously [30, 74] to predict fungal secretomes. Briefly, the pipeline filters a set of proteins that contain signal peptides using SignalP 4.1f [75]. This set of predicted secreted proteins were further used in the pipeline to predict transmembrane helices with TMHMM 2.0c [76] and cellular localisation signals with TargetP 1.1b [77]. Sequences that contained transmembrane helices or mitochondrial targeting signal were removed from the set of interest. The remaining proteins were annotated with: Hmmer [78] against PfamScan [79] for domain information, Targetp [80] for subcellular localisation, Predictnls [81] for detection of nuclear localization signals, T-Reks [82] for repeat identification, Disulfinder (database: uniprotkb/swiss-prot) for disulfide bond prediction, MOTIF search for the search of known motifs. The positions of the motifs RxLR, [LI]xAR, [RK]CxxCx{12}H, [YFW]xC, YxSL[RK], G[IFY][ALST]R, DELD and [SG]PC[KR]P are identified with a script based on regular expressions.

## Homology detection and structure prediction

To search for homologues and compare their predicted structure, a workflow that contains the non-modelling steps of the hhpred-website [83, 84] was implemented. The sequences were scanned with hhblits against uniref30 and pfam to obtain similar sequences, multiple alignments and hmms of the hits and the query sequences were generated, followed by a search of the hmms in pdb with hhsearch. Data were then extracted from hits (see S4 Table) and used for the analysis.

## Supporting information

### S1 File. Genome annotation.

(PDF)

### S2 File. EffectorP2 predictions.

(PDF)

### S3 File. SIX effector candidates.

(PDF)

### S4 File. SGPCKRP effector candidates.

(PDF)

### S1 Table. Secretome annotation.

(XLSX)

### S2 Table. CAZymes prediction.

(PDF)

### S3 Table. Cysteine-rich proteins.

(PDF)

### S4 Table. HHpred-SGPCKRP predictions.

(XLSX)

**S1 Fig. Geographic distribution of the date palm dieback disease.** Map was developed using ArcGIS 10.8 with layer from natural earth dataset. <http://www.naturalearthdata.com/>.

(TIF)

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