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Article

Seed Tubers Are Not the Primary Inoculum Source in Water Yam (*Dioscorea alata*) Anthracnose Epidemics in the Caribbean

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Abstract: Crop disease often leads to field epidemics with serious threats to yield. Early symptoms are sometimes difficult to identify, so the origin of primary inoculum is a critical focal point in the study of plant diseases, as it can help design management strategies to reduce crop losses. Here, we investigated whether anthracnose of water yams (*Dioscorea alata* L.) caused by the species complex *Colletotrichum gloeosporioides* can start from infected seed tubers from the previous harvest. Over two years, we collected tubers with varying pathogen prevalence in the field directly from producers and conducted fungal isolations in the lab to sample *C. gloeosporioides*. We also proceeded to artificially inoculate tubers before planting and monitored disease development. Finally, we genotyped isolates from leaves in the fields and assessed fixation indices between plots based on plot ownership (plots with a common seed tuber origin from a single farmer) vs. samples in plots from unrelated producers in Guadeloupe, Martinique, and Barbados. We were unable to isolate the fungus from harvested tubers in either sampling survey nor did any plants grown from inoculated tubers develop any disease symptoms during growth. Also, the genetic structure of samples within each plot was independent of plot ownership, though this occurred with varying levels in the different islands. These results suggest that contaminated planting material from seed tubers is not the primary source of the disease, which is in contrast to the common perception of yam anthracnose prevalence in the Antilles.

Keywords: anthracnose; *Colletotrichum gloeosporioides*; primary inoculums; infected tubers; *Dioscorea alata*



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1. Introduction

Plant diseases pose significant challenges to food production, often leading to reduced yields [1,2]. This reduction can occur directly through damage to harvested produce or indirectly by compromising the plant's efficiency in producing reserve organs, fruits, or seeds. Disease thus potentially impacts yield anytime, from planting and growth, to reproduction and fruiting, or even during the post-harvest storage season. While the overall effect might be negligible if only a few plants suffer from pathogen attacks in the fields, there are usually more dramatic impacts when the disease progresses through a cultivated plot to reach an epidemic stage. Disease control methods, such as crop rotation and the use of certified disease-free seeds, are commonly employed agronomic practices to reduce the risk of disease development in crops [3]. Sometimes, fungal epidemics result from aggravated disease levels on a regional scale [4,5] which dramatically increases the chance of local disease initiation [6]. The spread of disease is therefore more challenging to manage, as fields have recurrent spore inflows [7,8]. Most often though, lower and irregular inoculum pressure make it essential to spot disease initiation in the fields before a more serious spread threatens crop production locally.

Epidemic control strategies will address pathogen dispersal in two ways, and both rely on identifying the source of disease initiation from field inoculum sources. First, whenever diseases are already increasing locally and about to become regional pandemics, then most proximal inoculum sources will originate from neighboring fields with the same cultivated crop, a close relative [9,10], a similarly susceptible cultivated plant species [11–13], or even natural vegetation or weeds [14,15]. At the epidemic stage, successful control often relies on chemical intervention unless resistant varieties are available [16,17] or sometimes via defoliation management (e.g., [18]) or when plot edges are acting as efficient propagule barriers [19,20]. Indirect control can also occur at the landscape level, where field crop mosaics can buffer pathogen spread [21,22]. The occurrence of epidemics is often critical, so disease management depends on pathogen and disease monitoring programs [23] and integrated information flow [24,25]. Second, and probably a more frequent situation, is the onset of disease without sustained propagule dispersal over fields (i.e., beyond regional pandemic situations), and in this case, identifying inoculum sources before disease propagation is the primary consideration for successful disease management. Inoculum sources are thus an important issue in phytopathology, and many studies emphasize the need to identify them and alter agronomic practices to decrease disease risk [26].

Primary inoculum sources often depend on the existence of reservoirs in wild species or a neighboring field (either immediately adjoining or at a greater distance, depending on pathogen dispersal skills) [9,11], and in this case, the correct knowledge of potential alternative host species is necessary [15,27]. A second approach to identifying primary inoculum sources is to monitor crop species. The disease may start early during growth when a pathogen has already infected seeds [28,29]. The likelihood of disease development increases when producers have to produce seeds themselves as it depends on disease impacts from the previous cultivation season. Such is the case of our study model in Guadeloupe, since water yam (*Dioscorea alata*) seed availability is directly self-managed by producers, even if ancient and new varieties are available to renew their stocks [30] despite labile yield levels making cultivar match with local conditions difficult [31]. The main fungal disease in yam in the Caribbean is anthracnose, which is caused by the worldwide pathogenic species complex *Colletotrichum gloeosporioides* [32]. Yam anthracnose is a major threat locally, yet the disease occurs erratically and is difficult to predict despite a high prevalence of fungi in natural vegetation [14] and a year-round abundance of spores in cultivated areas [7]. So, the actual inoculum source for *C. gloeosporioides* causing anthracnose disease on yams remains uncertain.

Indeed, while the most frequent path to disease is probably a local inoculation from rain splash spread in the fields [33], *Colletotrichum* spp. are still thought to be able to infect tubers either directly by systemic infection during tuber filling or from fungi via direct soil contamination or indirectly at the harvest stage from diseased plant aerial parts such as necrotic leaves and stems. This narrative that yam anthracnose disease starts in infected fields produced several studies investigating the long-term survivability of the fungus and its infection capacity, which seems low in soil conditions [34,35]. Yet, while some producers seem reluctant to plant their own seed tuber material when disease prevalence was substantial in their fields in a previous cultivating season [36], a majority still acknowledge planting seed tubers independent of previous disease assessments. *Colletotrichum gloeosporioides* was previously described as a yam tuber pathogen, causing anthracnose in aerial parts and “dead skin” disease on tubers [37,38]. Some researchers hypothesize that infected tubers might be the primary inoculum source for anthracnose because developing plantlets from inoculated tubers develop symptoms typical of anthracnose disease [39], an idea recently updated by Frézal et al. [39,40]. Consequently, the hypothesis of tuber infection still drives disease levels in the fields [40], which is a process corroborated by the prevalence of disease spread via seed networks [41], as casual, informal seed exchange between acquaintances occurs frequently enough in the region.

In this study, we investigated whether yam seed tubers could act as primary inoculum sources yearly since producers believe that tuber contamination before planting was the

primary source and initiator of yam anthracnose in their fields [36]. There is earlier evidence that *Colletotrichum* spp. can infect yam tubers [37,38]. Nonetheless, *C. gloeosporioides* demonstrates high genetic diversity often at field levels [40,42]; thus, evidence might be pointing to other sources for inocula initiation (e.g., neighboring vegetation), though it is still required to test whether the genetic similarity of isolates is greater within fields from the same producers compared to genetic similarity from random fields. During the interview survey in 2014, many producers were interested in whether *C. gloeosporioides* isolates contaminated their seed tubers, as they generally thought these were primers in disease onset in the fields [30]. The research objectives can be written as follows: determining the prevalence of the fungus *C. gloeosporioides* in harvested tubers and investigating whether inoculation of fresh seed tubers leads to disease development during plant growth. Last, we tested whether tuber infection was associated with field ownership between post-harvest and pre-planting isolates. Indeed, if disease initiation occurs via tuber contamination, a greater genetic similarity would be expected with field isolates from the same producers than between isolates from randomly chosen fields. We tested this hypothesis with some yam producers who owned several yam fields and analyzed fixation indices of isolates for five microsatellite loci.

2. Materials and Methods

2.1. Tuber Sampling Surveys and Fungal Isolations

Seventy-eight Guadeloupean yam producers were interviewed to assess anthracnose disease control strategies and varietal dynamics with anthracnose epidemics [30,36]. In a first trial phase to grossly estimate the prevalence of *C. gloeosporioides* on yam tubers in the fields, 21 seed tuber samples were collected from 7 commonly cultivated varieties (Belep, Boutou, AnBa Bon, Goana, Kabusah, Kinabayo, Pacala, and Saint-Vincent) from a subsample of 18 producers from the initial study sample, all entirely volunteering for disease checks (usually with a single seed tuber, except for one producer who furnished five tubers from five different varieties).

In 2015, the tuber-checking survey for the prevalence of *C. gloeosporioides* was conducted with a larger group of farmers and with a greater agro-diversity-oriented sampling scheme, still relying on volunteer participation. Yet, it offered producers financial compensation equivalent to the market price of tubers received. We specifically sampled tubers in various states of health, harvest, and general shape or conditions (including spoiled or decaying ones), although the tubers were of good quality on average. The 2015 survey allowed sampling to a greater extent, and 213 tubers from 7 varieties from 50 producers were sampled. During the second survey, we decided to focus on *Colletotrichum* spp. only and did not proceed to identify any other fungi growing in culture. In the lab, we surface sterilized tubers in an external bath of one minute in 1% sodium hypochlorite and then 70% alcohol solutions, which was followed by two rinses in sterile distilled water. We then cut small slices of tubers with both pieces of epiderm and deeper layers intact, which were then placed in Petri dishes with S media [43] to ensure a more favorable growth condition for *C. gloeosporioides* [11]. Petri dishes were sealed for up to five days at room temperature, and we assessed fungi growing out of the yam pieces (ranging from skin and cortical zones to regions slightly deeper in the tuber) under light microscopy to assess the presence of *C. gloeosporioides*. Various minor modifications to the protocol were made, using a more general culture media (e.g., potato dextrose agar [44]), changing the size of tuber fragments, or increasing moisture content in Petri dishes during incubation to increase the chances of isolating *Colletotrichum* spp. in sampled tissues (see Results section). Our sampling scheme was fairly small in the first year (trial phase) and increased about ten times the second year on the 8 commonest varieties grown locally, representing over 85% of local varietal diversity in importance [30], and reaching about 3% of the island declared yam producers. Sampling was overall a fair and decent approximation of the local situation and context (dominance of family agriculture) even if it could have been improved in terms of robustness and size.

2.2. Tuber Inoculation and Greenhouse Experiment

In parallel, a greenhouse experiment was conducted where seed tubers from two widely grown varieties (“Plimbite,” a susceptible variety, and “Goana,” a moderately resistant one) were washed either for 10 minutes in a highly concentrated *C. gloeosporioides* spore suspension (10^5 spores·mL⁻¹, consisting of a mix of spores from two aggressive isolates #172 and #242 isolated from *D. alata* and kept at our lab isolate bank, i.e., inoculation treatment), or left as control for 10 minutes in a soap and water mixture, in a random fashion controlling for gross symmetric seed origin (head or low part of previous harvest tuber distributed symmetrically within treatments and pots). Our strains were confirmed to belong to *C. gloeosporioides* complex using CaInt2, CgInt and ITS4 primers (see below) [45]. Seed tubers were planted in pairs with eight replicate pots (i.e., two varieties by two treatments by eight replicates) in a spore-free room in the greenhouse (spore-free refers here to the filtering quality of the greenhouse netting), under natural light and ambient temperature (with a daily 24–32 °C range), and their growth was monitored as well as the appearance of potential disease symptoms. At the end of the growing season, mini tubers were harvested from the experimental pots and checked for the presence of *C. gloeosporioides* following the isolate sampling procedure described above. The experiment was replicated in the second year with the “Goana” variety only due to a shortage of “Plimbite” variety seeds with sample sizes twice that of the first year. Since there was strictly no difference between control and treatment in either year despite conditions highly conducive to infection (see results), power analysis could not be conducted, as it requires estimates of relative variance within plots and estimation of effect size. It was thus decided to estimate the infection threshold using binomial calculations. The individual probability of infection, ‘q’, was thus calculated. If $(1 - q)$ is the probability that the event would not happen (and the experiment was thus repeated $n = 64$ times, excluding controls), the following equation was solved $(1 - q)^n < \alpha$ (with $\alpha = 0.05$), i.e., $q > 1 - \alpha^{1/n}$ to determine q. The Wilson score formula was also used (see [46–48]) and gave the same result.

2.3. Genetic Structure and Plot Ownership

A broader *C. gloeosporioides* sampling survey was subsampled with identification based on spore morphology (more details discussed in [14]), following von Arx spore morphotypic classification [49] isolated from yam leaves that investigated the genetic diversity of the fungus in the Lesser Antilles [43], selecting fields from producers with 2–3 yam plots in their farms from three islands (Guadeloupe, Martinique, Barbados). DNA was extracted from the isolates using a FastDNA kit (MP Biomedicals, Irvine, CA, USA) and Lysing Matrix A for fungal cell lysis and then amplified via PCR the CaInt2, CgInt and ITS4 region [45] to confirm prior visual assessment by microscopy that the fungi indeed belonged to *C. gloeosporioides*. *Colletotrichum* spp. isolates sampled on yam leaves in 2014 were genotyped with five microsatellite markers, namely cg150, cg68, cg71, cg92, and cg164, following protocols described in [50]. A total of 109 isolates sampled from 21 yam fields from 8 producers from the three islands from the initial sample were thus subsampled. Barbados accounted for three producers with three plots each (9 plots total), allowing for 185 ‘inter’-comparisons and 40 ‘intra’-comparisons (225 single locus Fst estimates for Barbados Island). Martinique accounted for 2 producers with 2 and 3 plots, respectively, allowing for 30 ‘inter’-comparisons and 20 ‘intra’-comparisons (50 single locus Fst estimates for Martinique Island). Guadeloupe accounted for three producers with 2–3 plots (7 plots total), allowing for 60 ‘inter’- and 15 ‘intra’-comparisons (75 single locus Fst estimates for Guadeloupe Island). The hypothesis was that under a high prevalence of harvest and post-harvest tuber contaminations leading to using contaminated seeds as planting material, genetic similarity would be higher between fields from the same producers (intra) than between different producers (inter). Therefore, this would lead to a significant genetic structure of *Colletotrichum* spp. isolates based on producer location as a hierarchy factor. In contrast, if natural vegetation was the main factor in disease, we would expect no difference of genetic similarity for intra- and inter-comparisons. Single locus Fst between all pairs of

fields for all loci within islands (nested factor) were thus computed and arranged by type of comparison (intra vs. inter). It was then tested whether a significant difference existed between field pairs from the same producer (treatment comparison = intra) vs. field pairs from different producers (treatment comparison = inter) with an ANOVA via R software version 4.4.0 [51], using island and type of comparison (intra vs. inter) as independents.

3. Results

3.1. Fungal Diversity in Yam Tubers

The first tuber diagnosis survey in 2014 resulted in the identification of 19 different fungi genera based on spore morphology [52] at diverse occurrence on sampled tubers (number in brackets): *Aspergillus* sp. (9), *Bipolaris* sp. (1), *Cunninghamella* sp. (5), *Curvularia palesens* (4), *Curvularium eragrostidis* (1), *Drechslera* sp. (1), *Fusarium oxysporum* (3), *Fusarium roseum* (7), *Fusarium solani* (20), *Gliocladium* sp. (3), *Memoniella* sp. (1), *Mucoral* sp. (2), *Paecilomyces* sp. (3), *Papulorhiza* sp. (1), *Penicillium* sp. (6), *Phoma* sp. (1), *Pleurophagnium* sp. (1), *Rhizoctonia baticolla* (7), *Streptomyces* sp. (1), *Trichoderma* sp. (2), *Verticillium* sp. (10). *Colletotrichum gloeosporioides* was thus not found on or in sampled tubers from any producer among the 18 volunteers in the first year. Similarly, *Colletotrichum* spp. were absent on or in tuber slices in 2015 (though other species were isolated) (and thus with none of the 50 volunteers in the second year). *Colletotrichum* was thus never obtained as a sample isolate from tubers in the fields.

3.2. Infection of Yam Tubers with *Colletotrichum Gloeosporioides*

None of the greenhouse experimental plants growing from either control or tuber-inoculation treatment had any symptom of anthracnose from growth to senescence in either year. On the other hand, we used the same spore suspensions in an unrelated pathotyping experiment via regular leaf inoculation. They produced anthracnose symptoms on these plants, thus confirming that spores were viable and could infect plants naturally. Hence, the failure to infect tubers was not due to a protocol or pathogen viability issue.

The minimal infection threshold (q) fitting our model was approximately 0.047. We thus interpreted from our calculations that the fact that no infection occurred 64 times independently allows us to assume the probability of infection under highly favorable inoculation conditions is less than 4.7% with a confidence threshold of 95%. Using the probability of zero events in the binomial distribution, since none of the 64 plants was successfully infected, the 95% confidence interval of the infection probability lies between 0 and $-\ln(1 - 0.95)/64 = 0.047$. *Colletotrichum gloeosporioides*, along with *C. truncatum*, were isolated in both control and inoculation treatments at very low frequency (both *Colletotrichum* species were isolated twice for each treatment) after harvested tubers stayed in the lab, suggesting post-harvest contamination rather than infections resulting from the experimental inoculation. Both fungi are known to coexist at host level in nature and interact locally [53]. The greenhouse experiment demonstrated that tuber inoculation is not a primary factor of fungal contamination and disease initiation in the field. Consequently, tubers are not easily infected by yam anthracnose-causing fungi, so tuber contamination appears to be more of a post-harvest challenge than a problem for seed tubers.

3.3. Genetic Structure of Fungi Isolates within Plots

Pairs of F_{st} estimates were high because local sampled isolates often had alleles absent in other fields. Overall, the island was a significant factor for genetic differentiation (Table 1), but comparison treatment (intra vs. inter, i.e., fields from the same producers vs. fields from unrelated producers) was not (Figure 1). Thus, the evidence allows us to conclude that isolates sampled from fields with the same producers are not more similar than isolates from unrelated fields, further dismissing the hypothesis that contaminated seed tubers are the main path to anthracnose disease in yam plots in the Caribbean.

Table 1. Test of genetic structure (Fst comparisons) for isolates from plots within and between owners. Fst levels are significant between islands but not for treatment (comparisons of plots from the same owner vs. plots from different owners). *** indicates significance levels with $p < 0.001$.

	Df	Sum of Squares	Mean Square	F Value	<i>p</i>
Island	2	0.00484	0.0024213	8.324	0.000295 ***
Comparison	1	0.00001	0.0000062	0.021	0.884459
Island × Comparison	2	0.00000	0.0000011	0.004	0.996125
Residuals	344	0.10007	0.0002909		

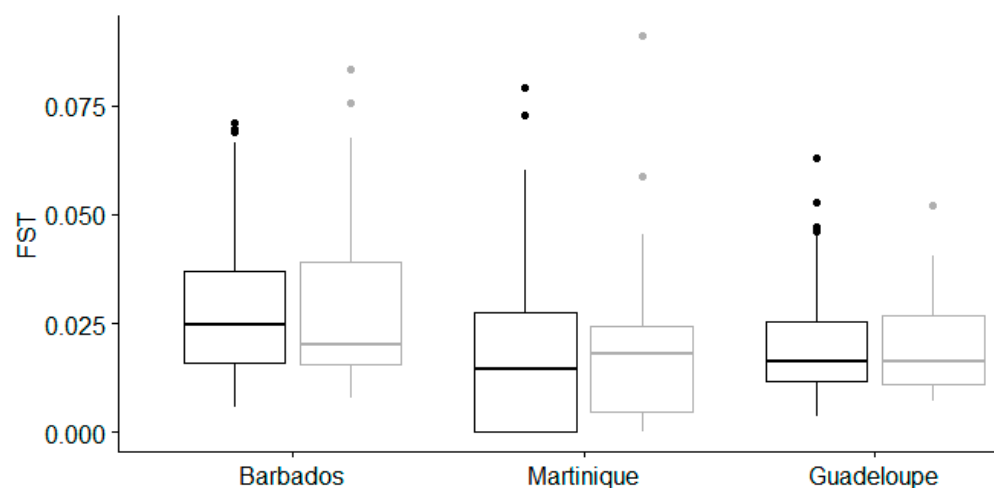


Figure 1. Structuration indices for isolates in plots from the same producers ('intra', gray boxes) vs. in plots from different producers ('inter', black boxes) in the three study islands (Barbados, Martinique, and Guadeloupe).

4. Discussion

Several genera of pathogenic fungi may infect yam tubers produced by farmers from Guadeloupe, as similarly indexed by Noon and Colhun in tubers from West Africa [54]. However, our results showed that anthracnose-causing *Colletotrichum* spp. did not colonize yam tubers (either external or internal areas) in this study. Also, mock-inoculated tubers did not produce diseased plants, but mock-inoculated yam leaves produced anthracnose symptoms. Still, *Colletotrichum* isolates can be isolated from yams in tubers post-harvest, possibly depending on the spore intensity in the environment and exposure to contaminated wasted leaf. Further, when we analyzed for possible genetic structural effects of plot location, there was no significant difference between plots from the same producers or unrelated fields, dismissing the idea that tuber infections might be an essential component of primary inoculum and the source of anthracnose disease or epidemics. Therefore, infected tubers do not necessarily translate into further diseased plants (unlike the more common leaf infections, which seem the natural path to yam anthracnose).

In our two-year study, we did not detect or isolate *Colletotrichum* spp. from tuber samples obtained from producers. So, we did not have any events of natural tuber infection (even after adjusting and expanding protocols to increase the probability of sampling *Colletotrichum* isolates). On the other hand, we successfully isolated casual *Colletotrichum* from our experimental tubers, which was most probably after exposure to a spore-contaminated environment (lab bench in our culture room). Thus, while tubers may host the fungus, naturally occurring infections of tubers seem rare. In addition, plants growing from experimentally inoculated tubers did not demonstrate any further symptoms of anthracnose disease in our greenhouse experiment, suggesting that local infection rates of tubers are probably insufficient at best to translate into disease. Most disease symptoms in the fields occur on leaves and very locally, and natural inoculation is mostly a matter of foliage transmission and dispersal (e.g., [55]).

On the other hand, there is little or no research on the influence of microbiota for protecting tubers during the post-harvest phase (i.e., the phase when they are likely most exposed to *Colletotrichum*), so this approach might still be worth investigating in small farms [56]. Contrary to regionally widespread views and as suggested by past evidence [38,39], seed tubers are not usually the primary inoculum source of anthracnose disease in the field. Our stratification study on the genetic diversity of fungal isolates has further confirmed this finding. Indeed, if tubers were the source of disease, then isolates sampled from fields from the same producers would be more closely related than fields from different producers, as many of them would come from a shared seed tuber pool (which would also share common inoculation events). In contrast, if local natural vegetation was the primary source of inoculum, we would not expect high similarity between samples from the same owners. Our results with Fst indices indeed demonstrated that the genetic similarity of isolates in fields from the same producer was not statistically different from those of randomly compared fields. The genetic diversity of isolates thus pointed more toward an extrinsic origin of the disease or environmental source [43] than an intrinsic origin such as seed tuber inoculation. A local vegetation component of inoculum source might explain this pattern better than seed tuber inoculation, which was indeed suggested by studies on host diversity patterns in both weeds [11] and natural vegetation [14], given the often observed lack of host specificity for *Colletotrichum* fungi [57].

Despite low tuber infection rates, *Colletotrichum* can contaminate and infect yam tubers. Indeed, we were successful in isolating samples from both *C. gloeosporioides* and *C. truncatum* in our yams from the inoculation experiment, though irrespective of experimental treatment (both inoculation and control), suggesting the contamination events were post-harvest (during storage in the lab culture room before processing). Unsurprisingly, these events would occur naturally in environments with high aerial spore charge. It may also occur whenever harvested tubers remain in the fields for too long next to potentially contaminated discarded aerial parts after harvest, especially if they show symptoms of anthracnose or if a ventilated stock room is located next to a potential contamination source (composted aerial plant parts that are diseased or alternate host growing in the neighborhood). Therefore, completely removing aerial crop residues is valuable in controlling for the inadvertent inoculation of yam tubers by *Colletotrichum* [36].

Our study results thus contradict previous studies on seed tuber inoculation as a potential source of primary inoculum in yams [37,38]. There may be several reasons for this disparity. First, a possible weakness of our isolation surveys might originate from underestimating actual inoculation rates because *Colletotrichum* spp. is less common on tubers, and they might escape sampling by chance (or due to protocol bias, or due to competitive isolation of other coexisting fungi). Since we isolated isolates from casual post-harvest contamination easily in the greenhouse experiment, this phenomenon probably occurs but would not fully account for the relative lack of *Colletotrichum* on yam tubers in our field study sample. Our sample covers most common locally grown varieties (representing about two thirds of yam varieties easily available in farms [30] and the majority of harvest since they are the most cultivated), and based on existing literature about soil survival of *Colletotrichum* [34,35] and our own experimental results on tuber contamination, we did not extend sampling survey. Even though our sample size is moderate overall (yet reaching out about 3% of local yam producers), with a total sample of 235 tubers from fields, we would have expected several positive isolations at a contamination rate of about 4.7% even if natural conditions proved less conducive than our favorable experimental conditions. The relatively small sample sizes in both years, even though it is based on the most commonly grown varieties accounting for nearly 80% of local yam cultivation, may limit the generalizability of the study findings, even if the results are congruent with both our inoculation experiment and our indirect evidence assessment via genetic diversity of the fungi. Second, disease expression on both seed tubers and sprouting plantlets can be very sensitive to the nature of isolates occurring locally in nature, and *C. gloeosporioides* with low infectious skills might not translate into disease, while more aggressive isolates would,

as suggested by the elective nature of specific minichromosomes in the species complex [58]. Still, we can hypothesize that the pathogen requires a more specific infection or different skill to infect tubers than aerial parts of the plant, notwithstanding that virulence genes have been readily identified for several *Colletotrichum* species (e.g., [59,60]). So, the results from this study would suggest that *Colletotrichum* infection is probably more of a matter of post-harvest inoculation, and the issue remains for tubers for sales, following other fungi attacking tubers post-harvest [61,62], and not seed provisioning. Unfortunately, control of post-harvest damage by *Colletotrichum* spp. is often more focused on vegetable fruit loss [63] than on yam tubers.

5. Conclusions

In summary, while yam tuber contamination by *Colletotrichum* is possible, it seems relatively uncommon and can be dismissed as the main factor in anthracnose epidemic development. Further research should investigate whether this result specifically depends on isolate virulence and aggressiveness, as these results contradict previous available evidence. Strongly differentiated isolates and high genetic diversity seem the norm in this species complex, even hinting at possible isolate specialization at the varietal level. However, this situation does not need to rely on the hypothesis that inoculated tubers are the source of disease in the fields, since plot ownership does not seem to correlate with the genetic structure of this pathogen. We provided evidence here that disease initiation from tubers is unlikely and that it is an entirely different issue from the inoculation competence of the pathogen even when considering low regional epidemics and propagule rains.

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References

1. Savary, S.; Teng, P.S.; Willocquet, L.; Nutter, F.W. Quantification and Modeling of Crop Losses: A Review of Purposes. *Annu. Rev. Phytopathol.* **2006**, *44*, 89–112. [[CrossRef](#)] [[PubMed](#)]
2. Zadoks, J.C. On the Conceptual Basis of Crop Loss Assessment: The Threshold Theory. *Annu. Rev. Phytopathol.* **1985**, *23*, 455–473. [[CrossRef](#)]
3. Berger, R.D. Application of Epidemiological Principles to Achieve Plant Disease Control. *Annu. Rev. Phytopathol.* **1977**, *15*, 165–181. [[CrossRef](#)]

4. Stone, L.; Olinky, R.; Huppert, A. Seasonal Dynamics of Recurrent Epidemics. *Nature* **2007**, *446*, 533–536. [[CrossRef](#)] [[PubMed](#)]
5. Nagarajan, S.; Kogel, K.H.; Zadoks, J.C. Epidemiological Analysis of the Damage Potential of Pgt-Ug99 in Central East, North East Africa; Iran and Punjab (India). *Indian Phytopathol.* **2014**, *8*, 26–32.
6. Wellings, C.R. Global Status of Stripe Rust: A Review of Historical and Current Threats. *Euphytica* **2011**, *179*, 129–141. [[CrossRef](#)]
7. Dentika, P.; Ozier-Lafontaine, H.; Penet, L. *Colletotrichum gloeosporioides*: Apparent Continuous Spore Rain May Hide Local Disparities and Iterative Disease Dynamics. *Trop. Agric.* **2022**, *99*, 333–340.
8. Lichtemberg, P.S.; Moreira, L.; Zeviani, W.M.; Amorim, L.; De Mio, L.M. Dispersal Gradient of *M. fructicola* Conidia from Peach Orchard to an Open Field. *Eur. J. Plant Pathol.* **2022**, *162*, 231–236. [[CrossRef](#)]
9. Parker, I.M.; Saunders, M.; Bontrager, M.; Weitz, A.P.; Hendricks, R.; Magarey, R.; Suiter, K.; Gilbert, G.S. Phylogenetic Structure and Host Abundance Drive Disease Pressure in Communities. *Nature* **2015**, *520*, 542–544. [[CrossRef](#)]
10. Suffert, F.; Sache, I.; Lannou, C. Early Stages of *Septoria tritici* Blotch Epidemics of Winter Wheat: Build-up, Overseasoning, and Release of Primary Inoculum: Primary Inoculum of *Mycosphaerella graminicola*. *Plant Pathol.* **2011**, *60*, 166–177. [[CrossRef](#)]
11. Dentika, P.; Ozier-Lafontaine, H.; Penet, L. Weeds as Pathogen Hosts and Disease Risk for Crops in the Wake of a Reduced Use of Herbicides: Evidence from Yam (*Dioscorea alata*) Fields and *Colletotrichum* Pathogens in the Tropics. *J. Fungi* **2021**, *7*, 283. [[CrossRef](#)]
12. Aylor, D.E. The Role of Intermittent Wind in the Dispersal of Fungal Pathogens. *Annu. Rev. Phytopathol.* **1990**, *28*, 73–92. [[CrossRef](#)]
13. Kazartsev, I.; Gomzhina, M.; Gasich, E.; Khlopunova, L.; Gannibal, P.B. Biodiversity of *Colletotrichum* spp. on Several Wild and Cultivated Plants. *Biol. Bull. Rev.* **2023**, *13*, S59–S70. [[CrossRef](#)]
14. Dentika, P.; Gumbau, M.; Ozier-Lafontaine, H.; Penet, L. Natural Flora Is Indiscriminately Hosting High Loads of Generalist Fungal Pathogen *Colletotrichum gloeosporioides* Complex over Forest Niches, Vegetation Strata and Elevation Gradient. *J. Fungi* **2023**, *9*, 296. [[CrossRef](#)] [[PubMed](#)]
15. Talhinhas, P.; Baroncelli, R. Hosts of *Colletotrichum*. *Mycosphere* **2023**, *14*, 158–261. [[CrossRef](#)]
16. Garrett, K.A.; Mundt, C.C. Host Diversity Can Reduce Potato Late Blight Severity for Focal and General Patterns of Primary Inoculum. *Phytopathology* **2000**, *90*, 1307–1312. [[CrossRef](#)] [[PubMed](#)]
17. Finckh, M.R. Integration of Breeding and Technology into Diversification Strategies for Disease Control in Modern Agriculture. *Eur. J. Plant Pathol.* **2008**, *121*, 399–409. [[CrossRef](#)]
18. Carisse, O.; Provost, C. Cluster Zone Leaf Removal Reduces the Rate of Anthracnose (*Elsinöe Ampelina*) Progress and Facilitates Its Control. *Plant Dis.* **2024**, *108*, 608–615. [[CrossRef](#)] [[PubMed](#)]
19. Dentika, P.; Ozier-Lafontaine, H.; Penet, L. Dynamics of Pathogenic Fungi in Field Hedges: Vegetation Cover Is Differentially Impacted by Weather. *Microorganisms* **2022**, *10*, 400. [[CrossRef](#)]
20. Gurr, G.M.; Wratten, S.D.; Luna, J.M. Multi-Function Agricultural Biodiversity: Pest Management and Other Benefits. *Basic Appl. Ecol.* **2003**, *4*, 107–116. [[CrossRef](#)]
21. Veres, A.; Petit, S.; Conord, C.; Lavigne, C. Does Landscape Composition Affect Pest Abundance and Their Control by Natural Enemies? A Review. *Agric. Ecosyst. Environ.* **2013**, *166*, 110–117. [[CrossRef](#)]
22. Plantegenest, M.; Le May, C.; Fabre, F. Landscape Epidemiology of Plant Diseases. *J. R. Soc. Interface* **2007**, *4*, 963–972. [[CrossRef](#)] [[PubMed](#)]
23. Van der Heyden, H.; Dutilleul, P.; Charron, J.-B.; Bilodeau, G.J.; Carisse, O. Monitoring Airborne Inoculum for Improved Plant Disease Management. A Review. *Agron. Sustain. Dev.* **2021**, *41*, 40. [[CrossRef](#)]
24. Sherman, J.; Burke, J.M.; Gent, D.H. Cooperation and Coordination in Plant Disease Management. *Phytopathology* **2019**, *109*, 1720–1731. [[CrossRef](#)] [[PubMed](#)]
25. Chades, I.; Martin, T.G.; Nicol, S.; Burgman, M.A.; Possingham, H.P.; Buckley, Y.M. General Rules for Managing and Surveying Networks of Pests, Diseases, and Endangered Species. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8323–8328. [[CrossRef](#)]
26. Filho, A.B.; Inoue-Nagata, A.K.; Bassanezi, R.B.; Belasque, J.; Amorim, L.; Macedo, M.A.; Barbosa, J.C.; Willocquet, L.; Savary, S. The Importance of Primary Inoculum and Area-Wide Disease Management to Crop Health and Food Security. *Food Secur.* **2016**, *8*, 221–238. [[CrossRef](#)]
27. Power, A.G.; Mitchell, C.E. Pathogen Spillover in Disease Epidemics. *Am. Nat.* **2004**, *164*, S79–S89. [[CrossRef](#)]
28. Cullen, D.W.; Lees, A.K.; Toth, I.K.; Duncan, J.M. Detection of *Colletotrichum Coccodes* from Soil and Potato Tubers by Conventional and Quantitative Real-Time PCR. *Plant Pathol.* **2002**, *51*, 281–292. [[CrossRef](#)]
29. Mancini, V.; Romanazzi, G. Seed Treatments to Control Seedborne Fungal Pathogens of Vegetable Crops. *Pest Manag. Sci.* **2014**, *70*, 860–868. [[CrossRef](#)]
30. Penet, L.; Cornet, D.; Blazy, J.-M.; Alleyne, A.; Barthe, E.; Bussière, F.; Guyader, S.; Pavis, C.; Péto, D. Varietal Dynamics and Yam Agro-Diversity Demonstrate Complex Trajectories Intersecting Farmers' Strategies, Networks, and Disease Experience. *Front. Plant Sci.* **2016**, *7*, 1962. [[CrossRef](#)]
31. Champoiseau, P.; Laurent, L.; Osseux, J.; Petro, D.; Tournebize, R.; Arnau, G.; Maledon, E.; Nudol, E.; Cornet, D. A Yam Collaborative Selection Platform in Guadeloupe: A Model for Effective Multipartenarial and Participative Program. In Proceedings of the Annual Meeting of Caribbean Food Crops Society, Guadeloupe, France, 10–16 July 2016.
32. Weir, B.S.; Johnston, P.R.; Damm, U. The *Colletotrichum gloeosporioides* Species Complex. *Stud. Mycol.* **2012**, *73*, 115–180. [[CrossRef](#)] [[PubMed](#)]

33. Penet, L.; Guyader, S.; Pétero, D.; Salles, M.; Bussière, F. Direct Splash Dispersal Prevails over Indirect and Subsequent Spread during Rains in *Colletotrichum Gloeosporioides* Infecting Yams. *PLoS ONE* **2014**, *9*, e115757. [\[CrossRef\]](#)
34. Ripoche, A.; Jacqua, G.; Bussière, F.; Guyader, S.; Sierra, J. Survival of *Colletotrichum Gloeosporioides* (Causal Agent of Yam Anthracnose) on Yam Residues Decomposing in Soil. *Appl. Soil Ecol.* **2008**, *38*, 270–278. [\[CrossRef\]](#)
35. Norman, D.J.; Strandberg, J.O. Survival of *Colletotrichum Acutatum* in Soil and Plant Debris of Leatherleaf Fern. *Plant Dis.* **1997**, *81*, 1177–1180. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Penet, L.; Barthe, E.; Alleyne, A.; Blazy, J.M. Disease Risk Perception and Diversity of Management Strategies by Farmers: The Case of Anthracnose Caused by *Colletotrichum gloeosporioides* on Water Yams (*Dioscorea alata*) in Guadeloupe. *Crop Prot.* **2016**, *88*, 7–17. [\[CrossRef\]](#)
37. Green, K.R.; Simons, S.A. “Dead Skin” on Yams (*Dioscorea alata*) Caused by *Colletotrichum gloeosporioides*. *Plant Pathol.* **1994**, *43*, 1062–1065. [\[CrossRef\]](#)
38. Adebajo, A.; Onesirosan, P.J. Surface-Borne Infection of *Dioscorea Alata* Tubers by *Colletotrichum gloeosporioides*. *J. Plant Prot. Trop.* **1986**, *3*, 135–137.
39. Frézal, L.; Jacqua, G.; Neema, C. Adaptation of a Fungal Pathogen to Host Quantitative Resistance. *Front. Plant Sci.* **2018**, *9*, 1554. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Frézal, L.; Desquilbet, L.; Jacqua, G.; Neema, C. Quantification of the Aggressiveness of a Foliar Pathogen, *Colletotrichum gloeosporioides*, Responsible for Water Yam (*Dioscorea Alata*) Anthracnose. *Eur. J. Plant Pathol.* **2012**, *134*, 267–279. [\[CrossRef\]](#)
41. Buddenhagen, C.E.; Hernandez Nopsa, J.F.; Andersen, K.F.; Andrade-Piedra, J.; Forbes, G.A.; Kromann, P.; Thomas-Sharma, S.; Useche, P.; Garrett, K.A. Epidemic Network Analysis for Mitigation of Invasive Pathogens in Seed Systems: Potato in Ecuador. *Phytopathology* **2017**, *107*, 1209–1218. [\[CrossRef\]](#)
42. Dentika, P.; Blazy, J.-M.; Alleyne, A.; Petro, D.; Eversley, A.; Penet, L. High Genetic Diversity and Structure of *Colletotrichum Gloeosporioides* Sl in the Archipelago of Lesser Antilles. *J. Fungi* **2023**, *9*, 619. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Messiaen, C.M.; Lafon, R. *Les Maladies des Plantes Maraîchères*; INRA: Paris, France, 1970.
44. Eddleman, H. *Bacteria Media from Potato*. Ph.D. Thesis, Indiana University, Bloomington, Indiana, 1998. Available online: <https://himedialabs.com/TD/M096.pdf> (accessed on 1 June 2024).
45. Liu, B.; Louws, F.J.; Sutton, T.B.; Correll, J.C. A Rapid Qualitative Molecular Method for the Identification of *Colletotrichum acutatum* and *C. gloeosporioides*. *Eur. J. Plant Pathol.* **2012**, *132*, 593–607. [\[CrossRef\]](#)
46. Wilson, E.B. Probable Inference, the Law of Succession, and Statistical Inference. *J. Am. Stat. Assoc.* **1927**, *22*, 209–212. [\[CrossRef\]](#)
47. Agresti, A.; Coull, B.A. Approximate Is Better than “Exact” for Interval Estimation of Binomial Proportions. *Am. Stat.* **1998**, *52*, 119–126. [\[CrossRef\]](#)
48. Tuyl, F.; Gerlach, R.; Mengersen, K. The Rule of Three, Its Variants and Extensions. *Int. Stat. Rev.* **2009**, *77*, 266–275. [\[CrossRef\]](#)
49. Von Arx, J. Kultur-Und Infektionsversuche Mit Einigen *Colletotrichum*-Arten. *Tijdschr. Plantenziekten* **1957**, *63*, 171–190.
50. Penet, L.; Briand, S.; Petro, D.; Bussi?re, F.; Guyader, S. Data on Microsatellite Markers in *Colletotrichum Gloeosporioides* s.l., Polymorphism Levels and Diversity Range. *Data Brief* **2017**, *12*, 644–648. [\[CrossRef\]](#)
51. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021.
52. Barnett, H.L.; Hunter, B.B. *Illustrated Genera of Imperfect Fungi*; Burgess Publishing Company: Menominee, MI, USA, 1960.
53. Dentika, P.; Ninsseu, A.; Pétero, D.; Ozier-Lafontaine, H.; Penet, L. Competition between *Colletotrichum* Species Reduces Anthracnose Symptom Development in *Dioscorea Alata* Yams: Potential for Biopriming and Breeding. *Plant Pathol.* **2022**, *72*, 442–448. [\[CrossRef\]](#)
54. Noon, R.A.; Colhun, J. Market and Storage Diseases of Yams Imported into the United Kingdom. *J. Phytopathol.* **1979**, *94*, 289–302. [\[CrossRef\]](#)
55. Martin, P.L.; Peter, K.A. Spore Dispersal Patterns of *Colletotrichum Fiorinae* in Orchards and the Timing of Apple Bitter Rot Infection Periods. *Plant Dis.* **2023**, *107*, 2474–2482. [\[CrossRef\]](#)
56. Hsieh, T.-F.; Shen, Y.-M.; Huang, J.-H.; Tsai, J.-N.; Lu, M.-T.; Lin, C.-P. Insights into Grape Ripe Rot: A Focus on the *Colletotrichum Gloeosporioides* Species Complex and Its Management Strategies. *Plants* **2023**, *12*, 2873. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Guevara-Suarez, M.; Cárdenas, M.; Jiménez, P.; Afanador-Kafuri, L.; Restrepo, S. *Colletotrichum* Species Complexes Associated with Crops in Northern South America: A Review. *Agronomy* **2022**, *12*, 548. [\[CrossRef\]](#)
58. Bhadauria, V.; Zhang, M.; Ma, W.; Yang, J.; Zhao, W.; Peng, Y.-L. The Hidden Truths of Fungal Virulence and Adaptation on Hosts: Unraveling the Conditional Dispensability of Minichromosomes in the Hemibiotrophic *Colletotrichum* Pathogens. *Int. J. Mol. Sci.* **2023**, *25*, 198. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Feng, L.; Dong, M.; Huang, Z.; Wang, Q.; An, B.; He, C.; Wang, Q.; Luo, H. CgCFEM1 Is Required for the Full Virulence of *Colletotrichum Gloeosporioides*. *Int. J. Mol. Sci.* **2024**, *25*, 2937. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Wang, D.; An, B.; Luo, H.; He, C.; Wang, Q. Roles of CgEde1 and CgMca in Development and Virulence of *Colletotrichum Gloeosporioides*. *Int. J. Mol. Sci.* **2024**, *25*, 2943. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Sánchez-López, D.B.; Luna-Castellanos, L.L.; Espinosa-Carvajal, M.R.; Pérez-Polo, D.J.; Cadena-Torres, J. Capacidad de Infección de Hongos Asociados a La Pudrición Seca de Los Tubérculos de Ñame. *Rev. Investig. Altoandinas* **2021**, *23*, 149–158. [\[CrossRef\]](#)

62. Anwadike, B. Fungal Rot of White Yam (*Dioscorea Rotundata*) Sold in Warri Markets, Nigeria. *Int. J. Agric. Res. IJAR* **2021**, *2*, 1–11.
63. Peralta-Ruiz, Y.; Rossi, C.; Grande-Tovar, C.D.; Chaves-López, C. Green Management of Postharvest Anthracnose Caused by *Colletotrichum gloeosporioides*. *J. Fungi* **2023**, *9*, 623. [[CrossRef](#)]

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