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# Scope, Distribution, and Cause of the Peanut Kernel Shrivel (PKS) Syndrome: An Emerging Threat to Australia's Peanut Industry

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**Abstract:** The cause of peanut kernel shrivel (PKS) syndrome, affecting peanut crops in Australia's growing regions, is currently unknown. It is estimated that PKS is costing the peanut industry more than AUD 5 M p.a. and is a potential threat to the industry. Previous investigations have ruled out all abiotic factors and most biotic factors as the cause of PKS. This research aimed at investigating the scope, distribution, and cause of the PKS syndrome. The survey showed PKS symptoms to be present in peanut crops in all the growing regions surveyed. Based on our study of culturable microorganisms, there appears to be no clear-cut involvement of plant pathogenic bacteria and fungi; however, *Fusarium* spp. were revealed as the most prevalent fungi in affected plants. Moreover, the soil metagenomics study revealed *Fusarium* spp. as the most abundant fungal communities in the soil microbial profile, and they could contribute to the PKS syndrome. The consistent presence observed of the identified *Fusarium oxysporum* in PKS-affected samples could indicate a role for this pathogen in the syndrome, especially in conjunction with abiotic stressors. The pathogenicity testing of *F. oxysporum* resulted in very mild PKS symptoms. A separate report suggesting the involvement of phytoplasma in the PKS syndrome raises the possibility of an interplay of biotic factors in the development of this disease. Further investigation is warranted to determine the true cause or causes of this disease.

**Keywords:** *Fusarium oxysporum*; Peanut Company of Australia (PCA); pathogenicity; Koch's postulates; soil metagenomics

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## 1. Introduction

The cause of peanut kernel shrivel (PKS), affecting peanut crops in Australia's peanut-growing regions, is currently unknown. It is a condition where kernels in some, or all, pods on a peanut plant approaching maturity cease normal development and fail to reach their full size, resulting in a low kernel percentage and high shell percentage, which reduces overall crop yield, quality/grading, and price/Mt of farmer stock [1]. Damaged kernels are smaller and have a shrivelled testa, while in more mature kernels, the testa appears faded due to a lack of assimilates from the plant and develops a brown/light tan colour. The 'funiculus' (or kernel umbilical cord), which feeds assimilates from the plant/pod to the developing kernel, often appears swollen, darkened, fibrous, and prominent compared to its smaller transparent appearance in a normally developing kernel. The swollen and unusual funiculus looks like it has resulted from some sort of 'physiological blocking' of assimilate flow from the plant to the developing testa/kernel. There are no other symptoms during vegetative growth, which appears quite normal, and the main quality constraint associated with PKS remains undetected until harvest. PKS does not affect peanut edibility or end-user traits; however, it has a significant impact on yield, kernel

grades, and returns, wherein the reduction in kernel size appears to be a result of the altered normal assimilate transport through the funiculus/testa [2]. Despite reduced size, kernels have normal blanchability (skin removal), taste and flavour, and germination. But with a lower percentage of larger peanut variety kernels and a higher percentage of kernels going to oil production, a significant financial penalty should be expected.

According to the Peanut Company of Australia (PCA), Kingaroy Australia, based on historic yield and grading information from Bundaberg and North Queensland over the past few years, PKS has cost the industry more than AUD 5 M, with reduced returns to growers in the order of AUD 500–1450/ha [2]. There has also been an indirect cost from the loss of confidence in growing peanuts in rotation with sugarcane in coastal production areas, and this has the potential to threaten the future viability of the Australian peanut industry. So far, PKS has been a bigger problem in coastal production areas around Bundaberg, even though it has been observed to a lesser extent in the inland Burnett region, southern Queensland, and North Queensland, and some years had a higher incidence than others [2]. Further, all the main commercially grown peanut varieties, such as Holt, Menzies, Kairi, and Taabinga, appear to be highly susceptible to PKS.

Preliminary investigations into the cause of PKS were conducted in 2015–2016, and the results have ruled out all abiotic factors (including water quality and nutritional status) and most biotic factors (including insects, nematodes, viruses, and bacteria). Research results at the University of Southern Queensland (UniSQ) and at the Commonwealth Scientific and Industrial Research Organisation (CSIRO)-Australian National University (ANU) have suggested that some sort of pathogen, e.g., fungi such as *Fusarium* and/or *Diaporthe* spp., may be involved (unpublished report). CSIRO has also suggested, from electron microscopic analysis, that symptoms expressed in the kernel (testa and funiculus) are consistent with a pathogen of some sort, and that the tissue in the swollen funiculus is quite typical of an excess of some kind of hormone.

According to Boote [3], co-author of a paper on the shrivelled trait in peanut [4], the genetic mutation causing shrivel in the University of Florida peanut mutant lines is not what causes the PKS symptoms observed in Bundaberg. So far, no one in the global peanut research community has seen PKS, but it raises concerns about this problem in Australian peanuts, with the threat that it might be capable of spreading to other countries. Sharman [5] suggested that PKS in peanut appears to be connected in some way to phytoplasma infection based on the observation that every peanut plant they received for testing that had phytoplasma symptoms tested positive for phytoplasma and had some or all kernels showing signs of PKS. Still, the role of soilborne pathogens in causing root rot, southern blight, crown rot, pod rot, and other root and stem diseases [6–8] cannot be discounted as a possible cause of or contribution to the PKS syndrome [9].

This investigation aimed to determine how widespread and severe the peanut kernel shrivel (PKS) syndrome is in the peanut-growing regions of Australia and to apply classical and modern procedures to identify the biotic cause of PKS.

## 2. Materials and Methods

*Peanut crop surveillance*. A total of 250 samples randomly selected from 82 crops in five peanut-growing regions in Australia were surveyed for PKS symptoms (Figure 1; Supplementary Table S1) [10,11]. Surveillance was performed between 14 and 20 weeks after planting, with three representative samples taken from each paddock and assessed for PKS symptoms. Peanut crop was considered as having PKS disease based on the following symptoms: peg lesions; yellow/swollen funiculus; discoloured testa; shrivelled kernel; and aborted kernels (Figure 2).

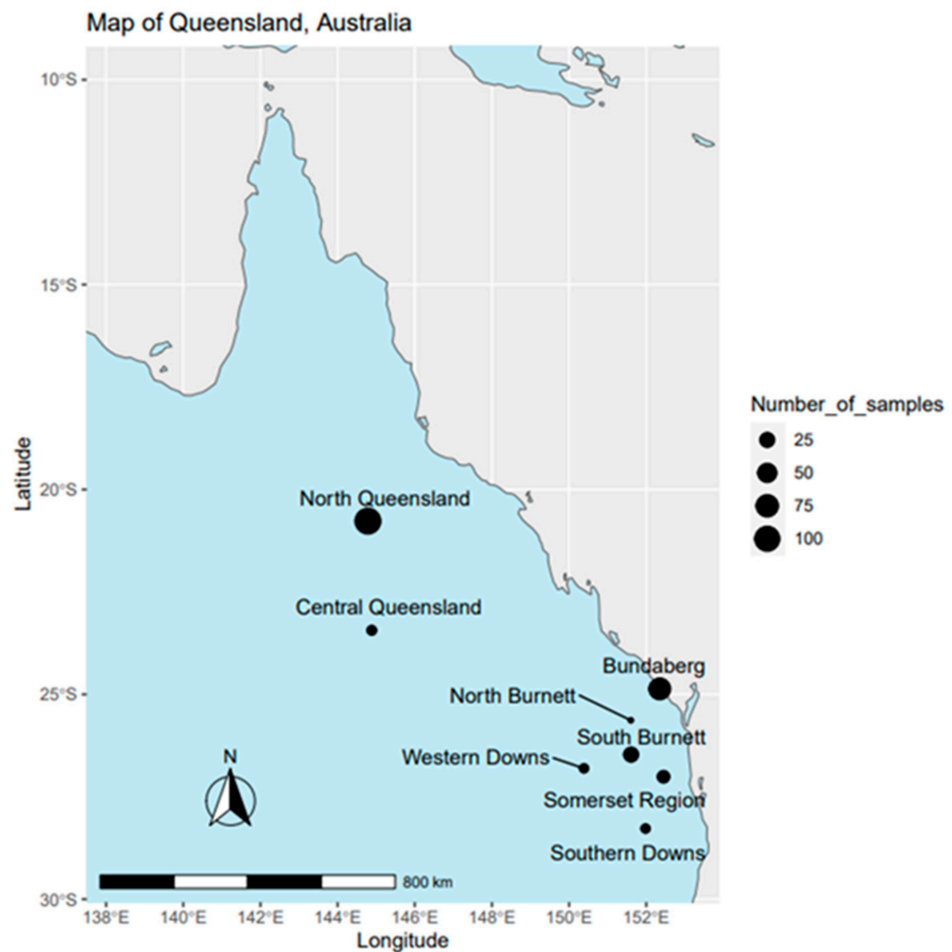


**Figure 1.** Number of samples from peanut crops surveyed in each peanut-growing region in Australia for 2016/17: Bundaberg, SQ = southern Queensland, CQ = Central Queensland, Burnett, and NQ = North Queensland, BUN = Bundaberg and BUR = Burnett.

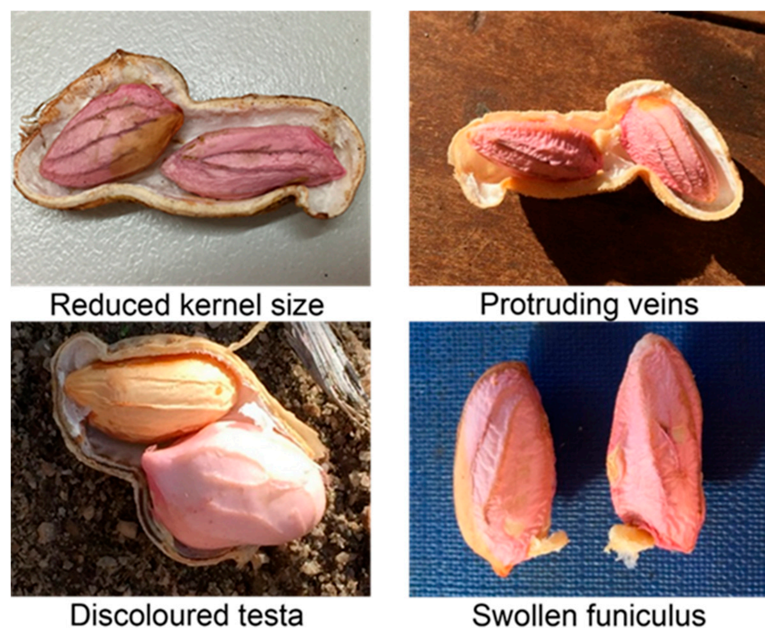


**Figure 2.** Symptoms of peanut kernel shrivel (PKS) syndrome.

*Fungi and bacteria isolation and identification*. A total of 77 PKS-symptomatic and -asymptomatic peanut plant samples from PCA's surveillance activity were used in the investigation. The samples represent nine peanut-growing regions, twenty-nine sample locations within the regions, seventeen peanut varieties, PKS-affected and -non-affected plants, twenty-five different planting dates, seventeen different sample collection dates, thirty different ages of plants (DAP—days after planting) when sampled, and sixteen different numbers of weeks when sampled.

*Morphology-based grouping*. Fungal and bacterial isolations on Potato Dextrose Agar (PDA) (Bacto Laboratories, Sydney, NSW, Australia) and Nutrient Agar (NA) (Bacto Laboratories, NSW, Australia), respectively, were performed separately on ten distinct parts of the peanut plant, namely, leaf, upper stem, middle stem, basal stem, peg, pod, funiculus, testa, kernel, and roots. All isolations were performed in three replications. Single spore isolation and hyphal tipping were performed on fast-growing and slow-growing fungi, respectively, to obtain pure cultures, while single colony sub-culturing was performed for bacteria. The isolated fungi and bacteria were characterised and grouped based on their morphology, examined by microscopy, and grown on culture media.

*Molecular identification*. For fungi molecular identification, total genomic DNA was extracted, and polymerase chain reaction (PCR) was performed using ITS1 and ITS4 primers on representative fungal isolates from each morpho-group, targeting the conserved Internal Transcribed Spacer (ITS) region for genus-level identification [12]. Sequencing was performed by Macrogen (Geumcheon-gu, Seoul 08511, Republic of Korea), and the resulting sequences were BLAST-searched in NCBI's GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (accessed on 15 January 2023) to obtain their closest identities at the genus level. For bacteria molecular identification, the universal primers 27F and 1492R were used for PCR to amplify the 16s rRNA region [13]. PCR products were sent to Macrogen (Macrogen Inc., Seoul, Republic of Korea) for sequencing, and the resulting sequences were BLAST-searched as described above. The identified fungal and bacterial genera from PKS-unaffected and -affected plants were compared. Those that were found consistently present and unique to PKS-affected plants were considered as a potential cause of the PKS syndrome. Cultures of the identified morpho-group representatives were used for further analysis as required.

For the species identification of the most dominant fungal genera, *Fusarium* spp., a partial sequence of the Translation Elongation Factor (TEF) *EF-1α* gene was obtained using EF-1 sequencing primer [14]. The *EF-1α* gene sequence data were used to determine the *Fusarium* species identity using the NCBI-BLAST (https://www.ncbi.nlm.nih.gov/nucleotide/) (accesses on 23 January 2023), Fusarium MLST Polyphasic Identification (https://fusarium.mycobank.org/page/Fusarium_identification) (accessed on 19 May 2024), and BOLDSYSTEMS (https://v3.boldsystems.org/) (accessed on 24 January 2023) platforms. A phylogenetic tree was created for the *EF-1α* gene sequences based on the genetic distance model, using the software Geneious Version 11 [15].

*Soil metagenomic analysis*. A total of 495 (165 × 3 replications) rhizosphere samples (a mixture of roots and soil), not necessarily all from the same locations where the peanut plant samples were collected, were received from PCA. The samples represent three peanut-growing regions (Brisbane Valley, Burnett, and Bundaberg), five crop rotations (no peanut, one season of peanut, two seasons of peanut, continuous crop, and pasture for 25 years), four peanut varieties (Holt, Fisher, Kairi, and Redvale), PKS-affected and -unaffected soil, fifteen paddocks, and fifteen sampling locations within a paddock. Roots and soil from each sample were separated, and then samples from the three replications were pooled to create a homogeneous mix. For the 165 soil samples, the Powersoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) was used for soil DNA extraction, while the CTAB DNA extraction method from the laboratory of Dr. Chris Dunlap of USDA, Illinois was used for the 150 root DNA extractions. DNA libraries were prepared and assays for both the fungal ITS and 16S rRNA gene sequence libraries were run in an Illumina MiSeq NGS machine using the Nextera XT v2 to generate FASTQ sequence data. The generated

FASTQ data were analysed using a CLC Microbial Genomics Module (Qiagen, Melbourne, QLD, Australia). Furthermore, a subset of the DNA samples was re-analysed using EF-1 fungal primers [16] to give a more definitive species assignment.

*Pathogenicity test*. A pathogenicity test was performed to determine whether the identified *Fusarium oxysporum*, found to be the most common and abundant fungal species of the PKS-affected plants and soil rhizosphere, would produce PKS symptoms. A "side-by-side" dual-pot experiment was conducted, using French white millet seeds soaked in distilled water for 24 h and autoclaved twice as the substrate inoculated with 9 mm mycelial discs of the *F. oxysporum* isolates and incubated at 25 °C for a minimum of three weeks, referred to from here on as the "*F. oxysporum* inoculum". Healthy peanut seeds cv. Taabinga were sown in the first 20 cm dia. plastic pot containing pasteurised potting mix (Searles Premium Advanced Potting Mix, Kilcoy, QLD, Australia), from here on referred to as "healthy soil". A second 20 cm dia. plastic pot containing the pasteurised potting mix was infected with the *F. oxysporum* inoculum at a rate of 10 g inoculum per kg of potting mix by mixing at the upper 5 cm layer of the potting mix, from here on referred to as "sick soil". No inoculum was added to the "healthy soil". The two pots were placed next to each other, which allowed the pegs from the "healthy soil" to extend and become embedded in both the "healthy soil" and the "sick soil" (Figure 3). After about 19 weeks, peanuts from the different treatments were harvested, and the pegs, pods, and kernels were inspected for presence of PKS symptoms.

**Figure 3.** The "side-by-side" dual-pot experiment with healthy peanut plant, cv. Taabinga, in pots containing "sick soil" (Left) next to the pot of "healthy soil" (Right).

## 3. Results

### *3.1. Peanut Crop Surveillance*

#### 3.1.1. Effect of Region

The Bundaberg samples had the highest incidence of symptoms, but also the highest variability in the symptoms that were detected. The Brisbane Valley samples had the lowest symptoms detected and the most uniform symptoms observed (Figure 4, Supplementary Figure S1).

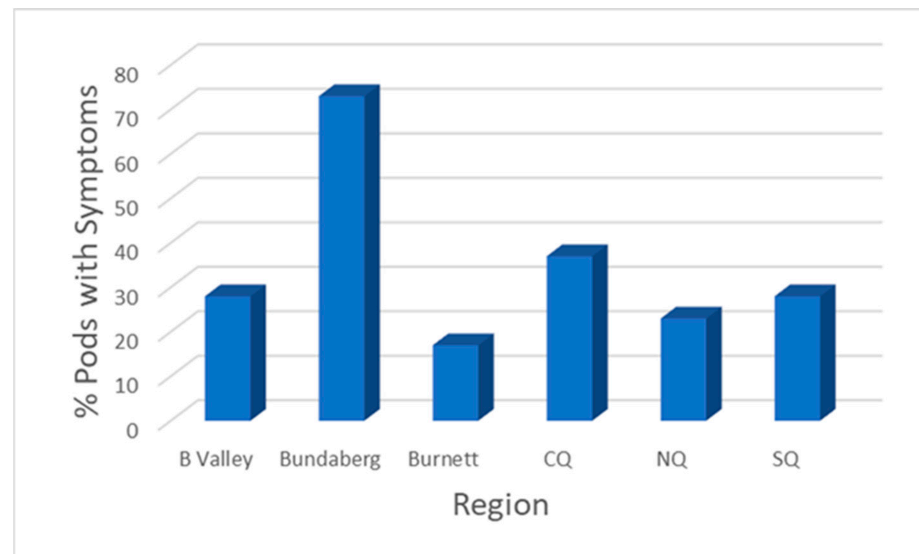


**Figure 4.** Percentage of pods showing PKS symptoms by region surveyed.

#### 3.1.2. Effect of Previous Crop

Results showed that Bundaberg with a previous crop of cane had the highest % of pods with PKS symptoms compared to the other previous crop, followed by peanuts, and then legumes at Central Qld (Supplementary Figure S2).

#### 3.1.3. Effect of Variety

Peanut cv. Holt at Bundaberg exhibited a higher % of pods with PKS symptoms compared to the other varieties (Supplementary Figure S3).

#### 3.1.4. Effect of Planting Date

Planting in October at Bundaberg resulted in a much higher % of pods with PKS symptoms compared to the other planting dates in the other surveyed regions, but a mid-January planting at North Qld followed next in % of pods with PKS symptoms (Supplementary Figure S4).

#### 3.1.5. Effect of Sample Days after Planting

Sampling between 100 and 140 days after planting resulted in a considerably high % of pods with PKS symptoms in all regions surveyed (Supplementary Figure S5).

#### 3.1.6. Effect of Soil Type

Alluvial soil and hydrosol in Bundaberg seem to favour PKS on pods, followed by ferrosol at NQ, and Tenosol at CQ (Supplementary Figure S6).

### *3.2. Fungi and Bacteria Isolation and Identification*

There were a total of 1560 fungal (42 morpho-groups) and 1560 bacterial (36 morpho-groups) isolations from 77 PKS-affected and -unaffected samples provided by PCA. Sequencing the 16S rRNA gene of bacteria morpho-group representatives resulted in bacteria belonging to the following genera: *Erwinia* sp.; *Pectobacterium* sp.; *Bacillus* sp.; *Pantoea* spp.; and *Serratia* sp. Furthermore, sequencing the *ITS1* gene of fungal morpho-group representatives revealed several fungal genus identities (Table 1). Comparing the fungi and bacteria isolated from above-ground and below-ground plant parts, between PKS-affected and -unaffected peanut plants, revealed no fungus or bacterium that was uniquely and consistently present in PKS-affected plants. This indicates that, based on culture-dependent samples, there is no clear-cut involvement of fungi or bacteria in the PKS syndrome. However, *Fusarium* spp. were found to be the dominant fungal genera in PKS-affected plants,

comprising 47% of the total number of fungi isolated from the surveillance samples (Supplementary Figure S7). Further amplification and sequencing of the *EF-1α* gene of the *Fusarium* spp. revealed that the most dominant *Fusarium* species is *F. oxysporum* (Figure 5).

**Table 1.** Identities of fungal morpho-group representatives based on *ITS1* gene sequencing and NCBI-BLAST search.

| Isolate ID# * | Morpho-Group | *ITS1* Region-Based ID | % Similarity |
|---|---|---|---|
| PKSF3 | A | *Fusarium* sp. | 99% |
| PKSF5 | B | *Fusarium* sp. | 97% |
| PKSF9 | C | *Fusarium* sp. | 100% |
| PKSF16 | D | *Fusarium* sp. | 100% |
| PKSF19 | E | *Fusarium* sp. | 100% |
| PKSF23 | G | *Diaporthe* sp. | 99% |
| PKSF29 | H | *Chaetomium* sp. | 99% |
| PKSF30 | I | *Alternaria* sp. | 99% |
| PKSF32 | K | *Alternaria* sp. | 99% |
| PKSF34 | M | *Paecilomyces* sp./*Talaromyces* sp. | 99% |
| PKSF37 | N | *Paecilomyces* sp./*Talaromyces* sp. | 99% |
| PKSF38 | O | *Penicillium* sp. | 98% |
| PKSF39 | P | *Chaetomium* sp. | 97% |
| PKSF40 | AI | *Fusarium* sp. | 99% |
| PKSF41 | D | *Fusarium* sp. | 99% |

* Isolates were curated at the University of Southern Queensland.

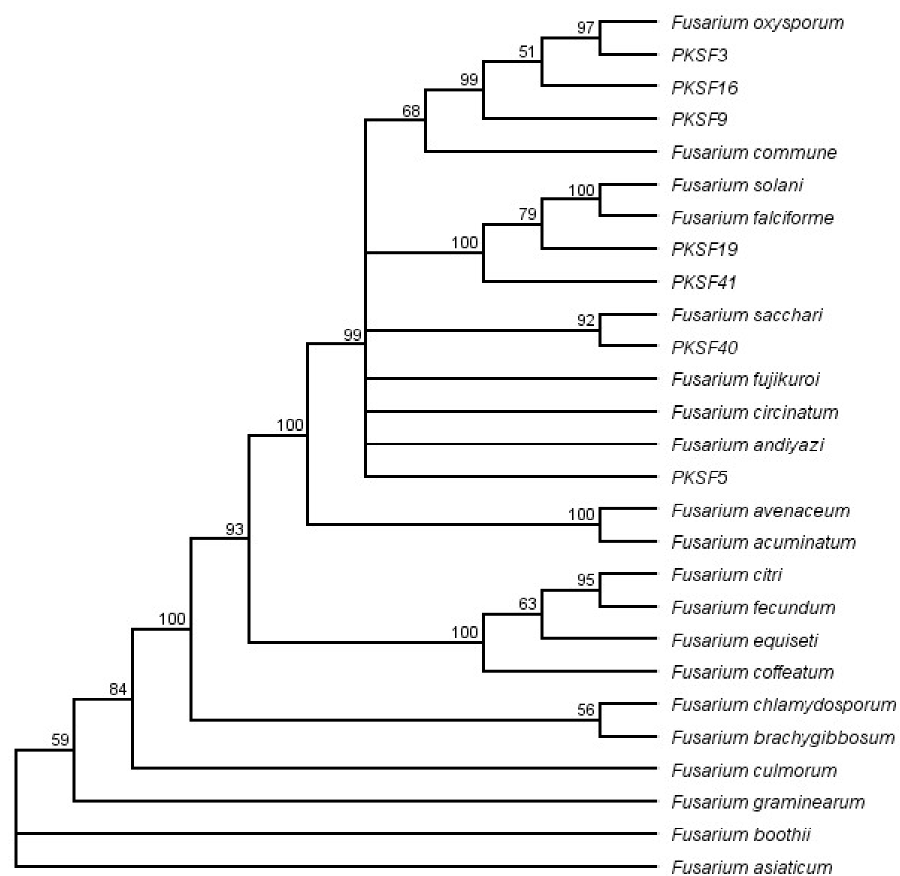


**Figure 5.** Phylogenetic tree of representative *Fusarium* sp. partial sequence of the Translation Elongation Factor (TEF) *EF-1* gene by genetic distance method.

*3.3. Metagenomics Based on Fungal Internal Transcribed Sequence (ITS)*

The Operational Taxonomic Unit (OTU) belonging to *Fusarium* spp. after blasting was found to be the most abundant. OTUs are clusters of (uncultivated or unknown) organisms, grouped by DNA sequence similarity of a specific taxonomic marker gene. Each of the formed clusters represent a taxonomic unit of a species or genus depending on the sequence similarity threshold. In this metagenomics study, all the graphs are at the genus level, and the OTUs for *Fusarium* spp. were made black in all the pictures. The unidentified sequences have been removed, and all graphs are a percentage of total fungi identified. There is one group of pictures for the soil and one for the roots. Each graph is the same data (all samples) grouped/sorted by the given variable.

3.3.1. Root Samples

Overall, *Fusarium* spp. detected from PKS-positive roots were more abundant compared to PKS-negative roots (Figure 6). Among the regions, Bundaberg was the region that had the most abundant *Fusarium* sp. communities, followed by Burnett, and then Brisbane Valley (Figure 7). In comparing the effect of crop rotation, there appeared to be a slight increase from 1 to 2 yrs. (Figure 8). From 0 yrs. to 1 yr. to 2 yrs., the Fusarium ITS increases from 6% to 12% to 15% of fungi, respectively. Among the varieties, Holt had the highest abundance of *Fusarium* spp. detected, followed by Fisher, and then Kairi.

3.3.2. Soil Samples

*Fusarium* spp. were more abundant in PKS-affected soil compared to PKS-unaffected soil. The Bundaberg region had the highest abundance detected, followed by Burnett, and then Brisbane Valley. Regarding the crop rotation effect, the results showed an increasing trend in terms of *Fusarium* sp. abundance from zero peanut, one peanut, and two peanut rotations (Figure 9). There is a difference in *Fusarium* sp. abundance in the soil of different peanut varieties, which follows the same trend as the root samples' results, with Holt having the highest abundance, followed by the Kairi and Fisher varieties.

*3.4. Metagenomics Based on Fungal Elongation Factor (EF-1)*

A subset of the root DNA samples that was re-analysed with EF-1 primer gave a more definitive species assignment. This allowed us to correlate the culture samples more easily to the metagenomics DNA samples. The reads came back as the *F. oxysporum* species complex after blasting with NCBI's GenBank database and the Fusarium MLST database. Matching these environmental *EF-1α* sequence data with the sequences from the culture isolates identified from the isolation and identification section of this project showed that the *F. oxysporum* species complex is the most common and abundant fungus of the PKS-affected plants and soil rhizosphere.

*3.5. Metagenomics Based on Bacteria 16S Data*

The 16S data did not show any meaningful results after a preliminary evaluation. No difference in bacteria communities and abundance between the PKS-affected and PKS-unaffected samples was revealed by metagenomics analysis.

*3.6. Pathogenicity Test*

After about 19 weeks, the pegs, pods, and kernels of harvested peanuts from the pegs that extended and became embedded in the "sick soil" displayed some of the peanut kernel shrivel (PKS) syndrome symptoms: peg lesions; shrivelled kernel; and aborted kernels (Figure 10).

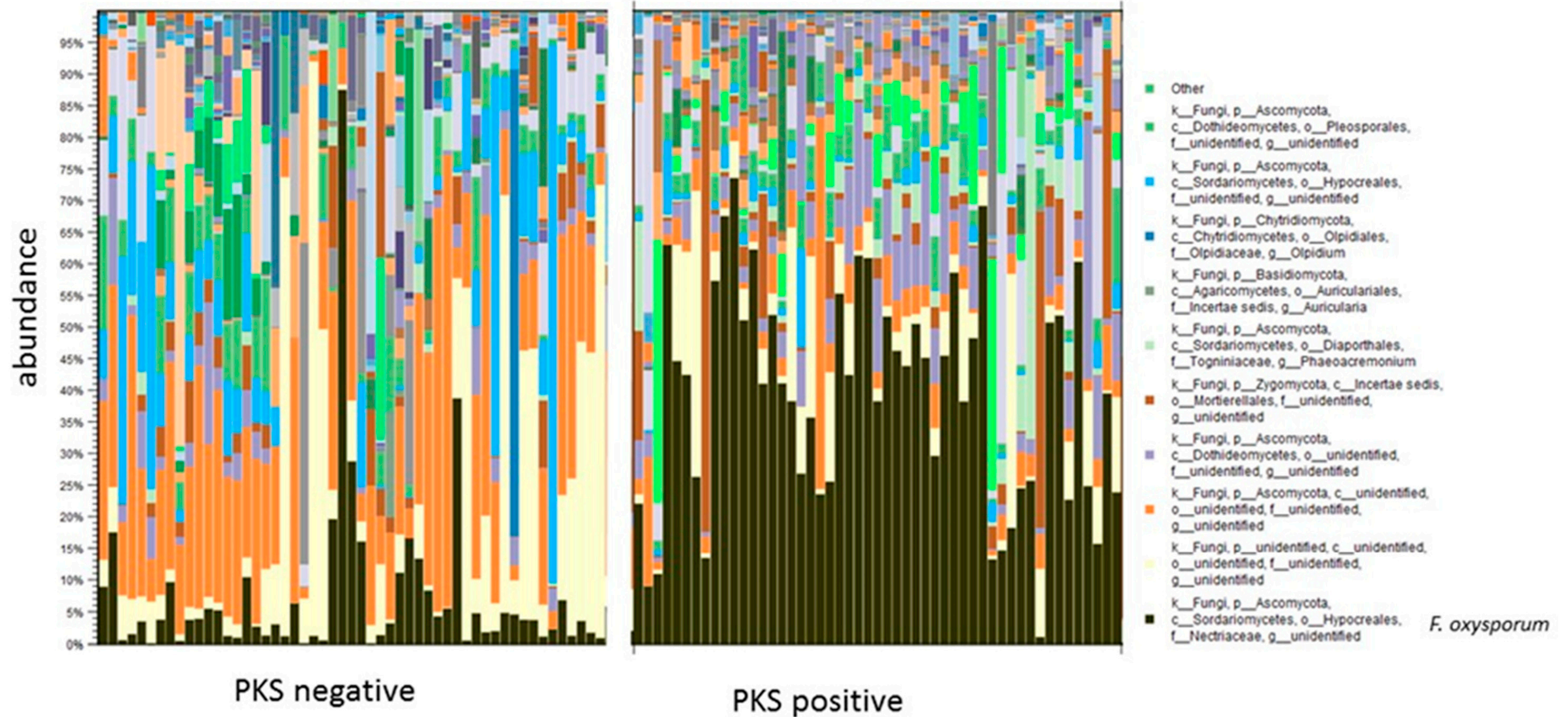


**Figure 6.** Fungal genera detected from PKS-positive and -negative roots. *F. oxysporum* in dark bars.

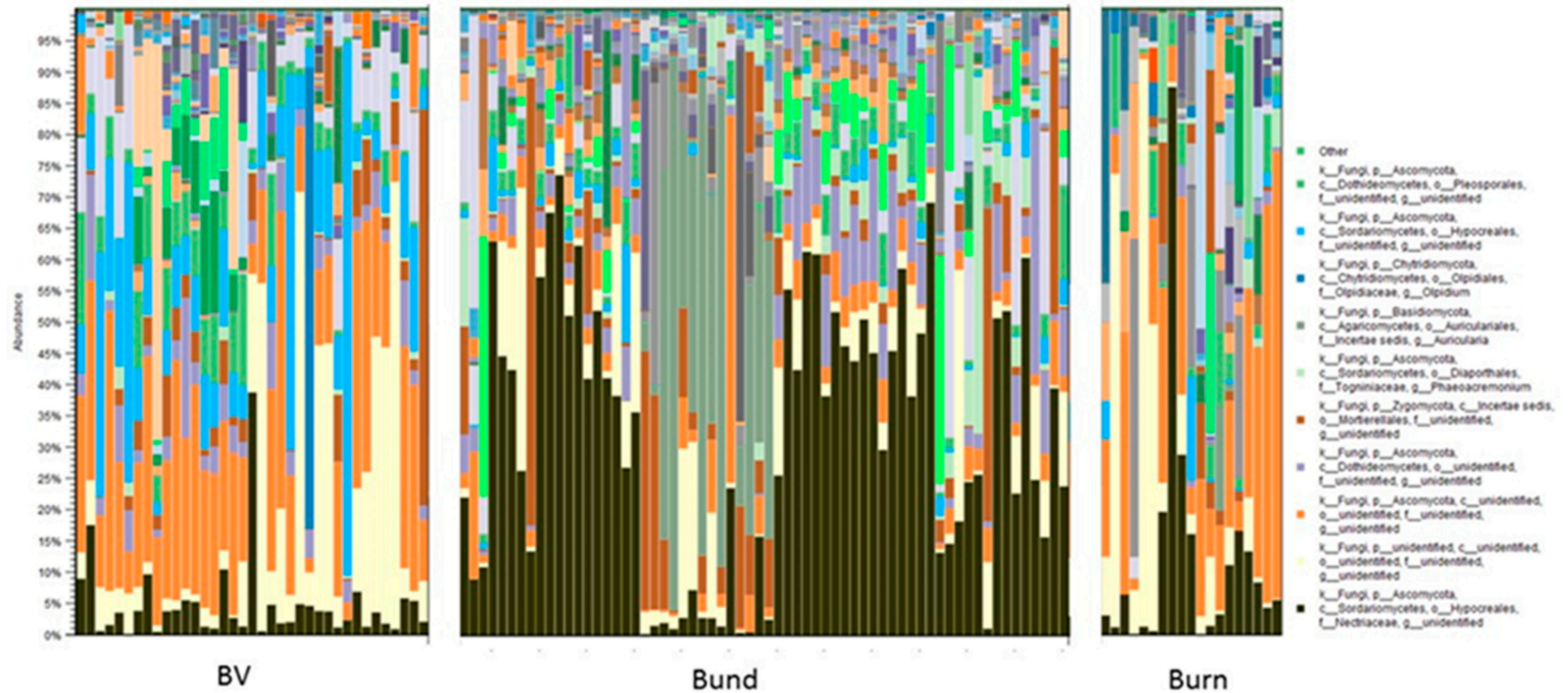


**Figure 7.** Fungal genera detected from root samples impacted by region: BV = Brisbane Valley, Bund = Bundaberg, and Burn = Burnett. *F. oxysporum* in dark bars.

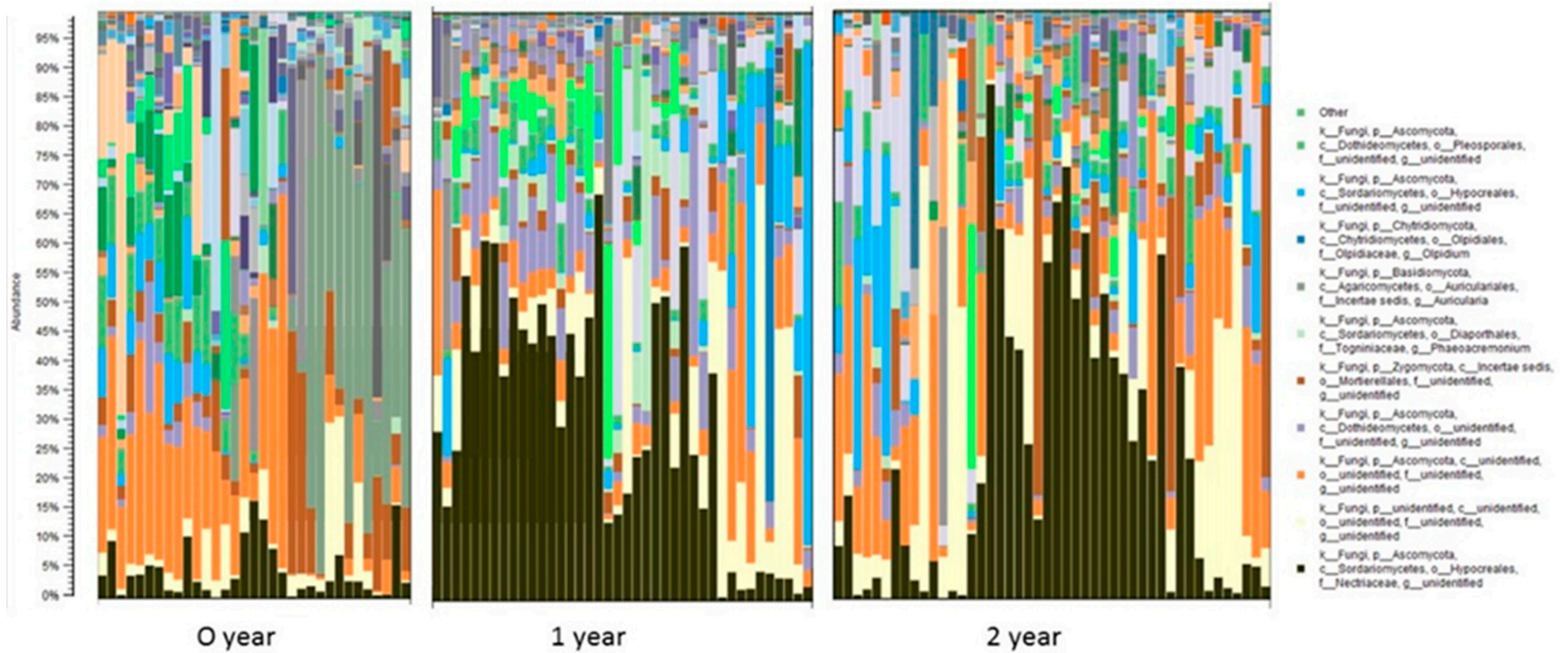


**Figure 8.** Fungal genera detected from root samples impacted by crop rotation. *F. oxysporum* in dark bars.

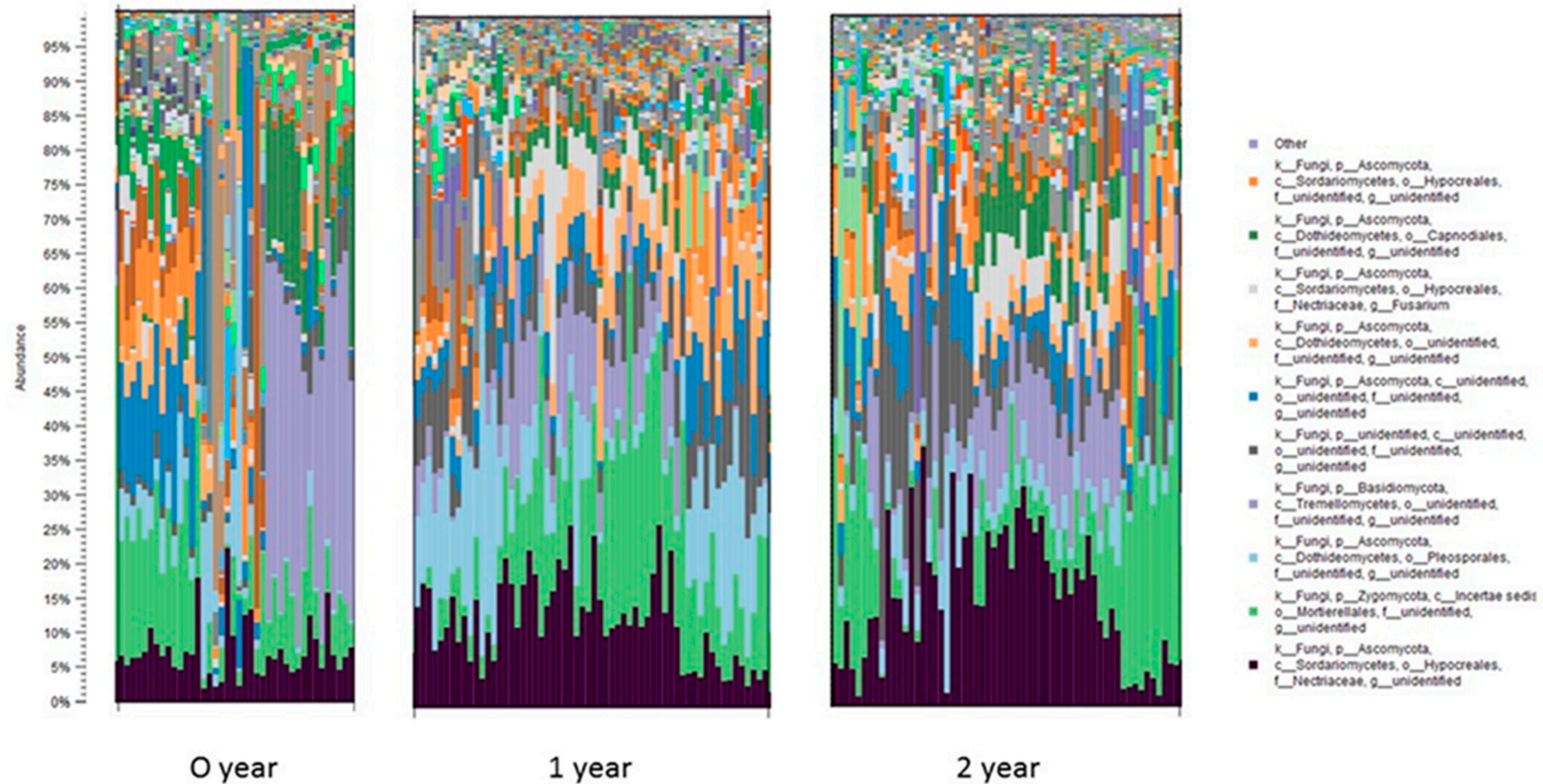


**Figure 9.** Crop rotation effect: results show an increasing trend in terms of *Fusarium* sp. abundance from zero peanut, one peanut, and two peanut rotations. *F. oxysporum* in dark bars.

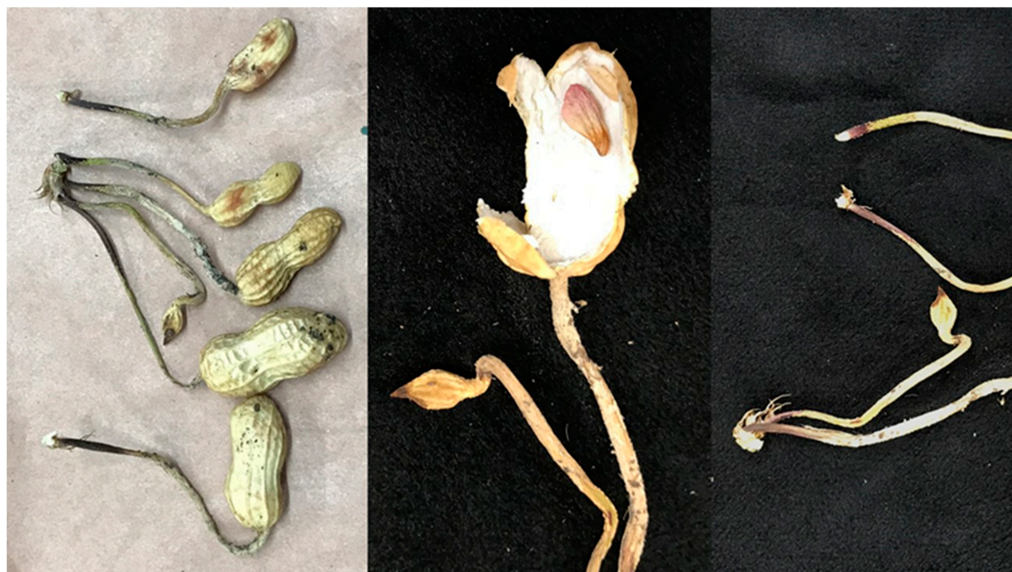

**Figure 10.** Result of the *Fusarium oxysporum* pathogenicity test, with peanut displaying some of the peanut kernel shrivel (PKS) syndrome symptoms: peg lesions; shrivelled kernel; and aborted kernels.

## 4. Discussion

The peanut crop surveillance conducted by PCA showed that PKS symptoms were present in all paddocks from the regions surveyed, indicating the possible threat that this syndrome brings to the Australian peanut industry. The most promising development to arise from the research undertaken in 2016 is the identification of two *Fusarium* species: *F. oxysporum* and *F. chlamydosporum*, which have been reported as being pathogenic to other crop species [17]. The present results agree with the result of the initial investigation at UniSQ, where the majority of fungi isolated from samples belonged to the *Diaporthe* and *Fusarium* species group [18]. CSIRO's electron microscopy results indicated that the tissue in the swollen funiculus is quite typical of an excess of some kind of hormone and is seen as a response to bacterial and fungal infection, and CSIRO-ANU also discovered two fungi which appeared to be unique to the infected kernels compared to the uninfected ones, namely, *F. oxysporum* and *F. chlamydosporum* [17]. According to CSIRO-ANU, *Fusarium oxysporum* is a prolific plant pathogen that is divided into distinct groups based on the host it infects. While *F. chlamydosporum* is far less associated with the disease, it is commonly found in soils and rhizospheres. It was suggested that *F. chlamydosporum* is unlikely to be the causal agent of the disease; however, it should not be excluded without further experimentation. There is also a possibility that it could form a part of a disease complex with *F. oxysporum* that could be causal to PKS. Furthermore, the results of the recently concluded bacterial and fungal isolations from plant samples received from PCA also showed *Fusarium* spp. as the dominant fungal genus present on PKS-affected plants. This agrees with the report of Bellgard and Ham [8] on the participation of *Fusarium* spp. in peanut peg and pod rot complex observed in the northern territory. The metagenomics study showed a significant difference in *Fusarium* sp. abundance between PKS-affected and PKS-unaffected samples, with *F. oxysporum* as the key *Fusarium* species [19].

*F. oxysporum* is a soilborne ascomycete common in soils around the world and the cause of fusarium wilt, a deadly vascular wilting syndrome in plants. It has over 120 known strains or "special forms" (*formae speciales*; f. sp.), each of which is specific and causes disease in a unique plant host. These *F. oxysporum* strains infect and kill a large host range, including many commercially harvested crops such as species in the Solanaceae family: tomatoes, peppers, potatoes, eggplant, lettuce, legumes, beets, basil, strawberries, chrysanthemum watermelon, sugarcane, bananas, and many other species [20]. *F. oxysporum* enters its host through the root where it grows in the xylem tissue, eventually blocking the vascular system. The blockage prevents the transport of water and nutrients within the host, causing

wilting, discoloration, and death of the plant (Gonsalves and Ferreira, 1993). Excessive soil moisture predisposes pods to infection by Fusarium and other pod-rotting pathogens, resulting in the death of very young pods and dry rot in mature pods [21]. Such events are typical of the PKS syndrome, which makes the identified *F. oxysporum* the probable cause of PKS. However, the pathogenicity test demonstrated that *F. oxysporum* alone did not produce the typical PKS disease symptoms. It is possible that it combines with other biotic and abiotic factors to produce typical PKS symptoms. In related investigations, the unpublished reports of Sharman [5] based on PKS surveillance and Vukovic, Sharman [22] based on graft- and leafhopper-mediated transmission of phytoplasma disease between peanut plants have suggested that PKS is a symptom of phytoplasma infection. This recent research has narrowed down the potential causes of PKS to an insect-vectored phytoplasma and/or the fungus *F. oxysporum* [9]. In addition, Laycock [23] also suggested that PKS has a strong interaction with seasonal environmental conditions. Unless the four criteria of Koch's postulates have been met to establish the true causal relationship between the pathogen(s) and PKS via individual inoculation or co-inoculation of suspected causal organisms, the cause of this syndrome will remain unknown. Nevertheless, as a precautionary measure, the presence of PKS should not be ignored, and measures to minimise it should be practised as a management strategy.

Once a field is infected with Fusarium, the infectious spores remain present in the soil for years. Crop rotation practices might help reduce the speed with which pathogen populations build in the soil but do little to reduce the number of infectious spores and eventually will not reduce the incidence or severity of the disease [21]. *F. oxysporum* is a soilborne pathogen, and currently controlling the initial inoculum by sanitation is the best means to control it. Moreover, the result of PCA's field experiment in Bundaberg indicated that there exists some level of resistance to PKS in some peanut varieties, wherein very large genotypic variation in PKS susceptibility/resistance in their variety trials was observed, suggesting that the genetic control over PKS resistance may be quite strong and offers the potential for genetic resistance as a longer-term management strategy for PKS [2]. There are reports as well from abroad on available peanut resistance to soilborne pathogens that could potentially be useful in breeding efforts to address PKS disease [24–27].

The findings of the current project indicated that *F. oxysporum* is the most likely cause of the PKS syndrome, in combination with other biotic and abiotic factors. Currently, controlling the initial inoculum by sanitation is the best means to control soilborne pathogens. One should avoid bringing infected soil or plant tissues into disease-free areas. Make certain that all tools and equipment have been cleaned and sterilised after contact with infected sites and plants. New plantings should be in areas known to be free of the pathogen, and the soils should be screened to ensure that no soilborne pathogen is present.

## 5. Conclusions

This investigation was able to demonstrate that *Fusarium* spp. were the most abundant fungal genus communities in PKS-affected plants and soil, with *F. oxysporum* identified as the most abundant *Fusarium* species. However, the pathogenicity test (Koch's postulates) for the fungus *Fusarium oxysporum* failed to demonstrate that it is the main cause of PKS, suggesting that it could just have an integral role in combination with other biotic factors and abiotic stressors to cause the disease. Further investigations are needed to identify the main cause or causes of this disease. This information can then be used by plant pathologists in developing disease management strategies, plant breeders in developing resistant/tolerant varieties, and the growers/peanut industry in implementing available disease management strategies.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14071435/s1, Figure S1: Effect of region on percent of pods with PKS symptoms; Figure S2: Effect of previous crop on percent of pods with PKS symptoms by region; Figure S3: Effect of peanut variety on percent of pods with PKS symptoms by region; Figure S4: Effect of planting date on percent pods with PKS symptoms by region; Figure S5: Effect

days to sampling after planting on percent of pods with PKS symptoms by region; Figure S6: Effect of soil type on percent pods with PKS symptoms by region; Figure S7: Percent distribution of different fungal genera representing different fungal morpho-group isolated from PKS surveillance; Table S1: GPS coordinates of sampling locations for peanut crops, including peanut variety information, surveyed in each peanut-growing region in Australia for 2016/17: Bundaberg, SQ = southern Queensland, CQ = Central Queensland, Burnett, and NQ = North Queensland.

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