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# Stigma and Stand Specificity in *Fusarium verticilloides* Associated with *Talbotiella gentii*

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## ABSTRACT

Pathogens, mainly fungus, were after fruit set, the most common cause of fruit abortion in *Talbotiella.* The measurement of stigmatic pollen load demonstrates that there is no shortage in the quantity of pollen flow within *Talbotiella* populations. However, germination of pollen grains on stigmatic surface of the species and growth of pollen tube down the style to fertilize ovules are inhibited by fungal hyphae. Air sampling at 3 populations of *Talbotiella* using 3 agar media was carried out to find out the prevalence of fungi. A total of 26 fungal species belonging to 17 genera was observed. The genera *Aspergillus*, *Penicillium* and *Cladosporium* recorded highest number of species. *Penicillium aurantiogriseum* and *Fusarium verticilloides* were the dominant species within *Talbotiella* populations. *Fusarium verticilloides* was found to mainly occupy the stigmatic surface of the *Talbotiella* species.

**Keywords:** air-borne, fungi, pathogenic, population, spores

## INTRODUCTION

*Talbotiella gentii*, a rare endemic species in Ghana, is under severe threat of extinction (Swaine and Hall 1981) therefore requires immediate conservation measures to prevent the few remaining populations from extinction. Though *Talbotiella* is a non-timber product in Ghana, it produces excellent charcoal of very high market demand therefore the species is preferentially exploited for charcoal. Periodic bush fires and farming activities have also contributed to the loss of *Talbotiella* populations. Little progress has been made in conservation of *Talbotiella* partly due to lack of information on its reproduction and genetic diversity. Information on the genetic diversity of the few isolated *Talbotiella* populations remaining will suggest which populations are more heterogeneous that could be targeted for *ex situ* conservation. To date, no integrated plan for conservation of *Talbotiella* has been developed though the Forestry Commission has made some effort to reduce its exploitation for charcoal and fuelwood. Records of *Talbotiella* are shown in **Fig. 1**.

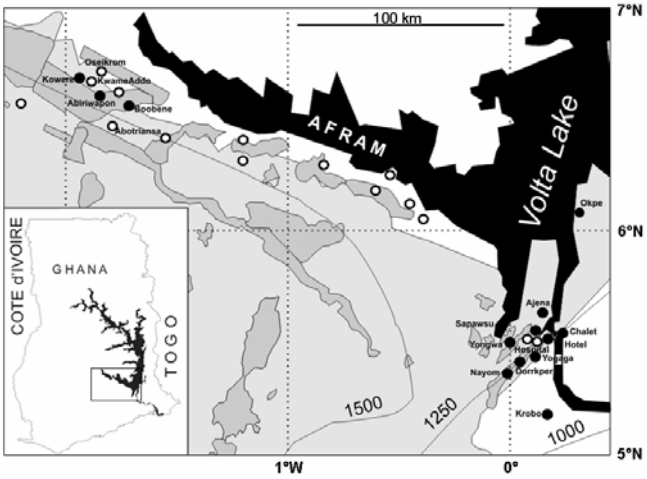


**Fig. 1 Records of *Talbotiella* populations in Ghana.** Filled dots = extant populations, empty dots = extinct populations.

The number of flowers initiated by plants normally exceeds the final number of mature fruits (Stephenson 1981; Bustan and Goldschmidt 1998) and fruit mortality due to internal (e.g. Nichols and Walmsley 1965; Udovic and Aker 1981; Pías and Guitían 2006) and external (e.g. Louda 1982; Arnold *et al*. 2003) factors can be a major drawback in plant reproduction. Results from reproductive studies of *Talbotiella* revealed that flowers and developing fruits are infested with spores and hyphae of fungi (Dompreh, unpublished). Pollen germination on the stigmatic surface may therefore be inhibited by fungal infection and could be the cause of pollination failure, low seed set or premature fruit abortion in *T. gentii*. Fungal spores which land on plants can infest flowers and leaves causing plant diseases or limiting light penetration and photosynthetic activities. Fungal infection may also inhibit fruit and seed development resulting in premature fruit and ovule abortion.

Flowers are considered excellent microbial habitats (Brysch-Herzberg 2004; Ngugi and Scherm 2006). They produce various types of nutrient-rich secretions (nectar, stigmatic exudates, and pollen exudates) that may serve as a substrate for colonization and infection by plant pathogens and saphrophytes. Plants produce flowers in exposed or elevated positions to facilitate pollen and /or seed dispersal. In out-crossing plants, increased height favours pollen dispersal by wind and insect pollinators (Carromero and Hamrick 2005; Rocha *et al*. 2005), therefore flowers are easily infested with fungal pathogens if present in the air. Flowers serve as a pathway for infection of the developing seeds and fruits, with associated benefits of nutrient supply for the fungus. Nectar is rich in nutrients and would therefore favour colonization and infection by plant pathogens and saprophytes (Brysch-Herzberg 2004; Ngugi and Scherm 2006). Thus, flower-infecting fungi can readily take advantage of the resources allocated to host reproduction. Flower-infecting fungi, by sporulating on the plant inflorescence,

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are also in an ideal position to facilitate their dispersal (Clay 1991; Antonovics 2005; Ngugi and Scherm 2006). The deposition of fungal spores on stigmatic surfaces of flowers may affect pollen performance by either physically reducing the amount of stigmatic surface area available for pollen germination since spores compete with pollen for germination space and /or by inhibiting pollen germination and tube growth down the style through chemical process.

Airborne pathogenic and saprophytic fungi may be of different types (species) and the level of pathogenic effect on host plants may differ from species to species. Some of the most common airborne fungi belong to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Candida*, *Rhodotorula*, *Trichoderma*, *Cephalosporium* and *Chaetomium.* Fungal spores in air, whether pathogenic or not, compete with pollen grains for space and nutrients on stigmatic surface, inhibiting pollen germination and may be limiting seed set in *Talbotiella.*

In the present study, aeromycoflora within the populations of *T. gentii* were sampled, isolated, cultured on agar media and identified. Fungi on the stigmatic surface (pathogenic or non pathogenic) surface of flowers of *Talbotiella* were also isolated and identified. Comparison of fungal infection rates with pollen germination may offer an explanation for differences in fruit abortion rates in *Talbotiella*.

## MATERIALS AND METHODS

### Study site

Three populations (Yongwa, Sapawsu and Hotel) were selected for the aeromycoflora studies in *Talbotiella.* Yongwa is about 17.5 and 16.2 km from Sapawsu and Hotel, respectively. Selection of these sites was based on the level of pathogenic fungal infestation of the *Talbotiella* flowers recorded in a reproduction experiment by Dompreh (unpublished) and the concurrent rates of fruit abortion in *T. gentii.* The study was carried out during the peak of flowering period of *Talbotiella.*

### Sampling of aeromycoflora at the three populations

Five 9-cm diameter replicate Petri dishes of three media dichloranglycerol agar (DG-18; Hocking and Pitt 1980), potato dextrose agar (PDA; Merck 2002), oxytetracycline glucose yeast extract agar (OGYE; Mossel *et al*. 1970) appropriate for the development of different fungal groups, and also to increase the chance of capturing many spores, were exposed to open air for 5, 10, 15, and 20 min at the three sampling sites. In all, 20 replicates (4 × 5) of Petri dishes per population were used. Dishes were covered, sealed with Sellotape and transported to University of Ghana, Legon mycology laboratory for isolation and identification. Samples were taken at a height of 1.5 m above the ground level in the middle of the populations and in addition five Petri dishes were placed 10, 20 and 30 m from all the three populations. Dishes were incubated at 28-30°C for up to 7 days and checked periodically. The resultant colonies at the end of the period were counted and identified. Counted colonies were expressed as colony forming units per plate per hour (cfu/p/h).

### Identification of fungi

The species of fungi isolated were identified by their morphological, colour and other cultural characteristics using standard and reference texts and identification manuals (Thom and Raper 1945; Smith 1960; Von Arx 1970; Barnett and Hunter 1972; Raper *et al.* 1973; Neergaard 1983; Klich and Pitt 1988; Samson and Reenen-Hockstra 1988; Samson *et al*. 2007).

### Isolation and identification of fungi on stigmatic surface of flowers of *Talbotiella*

Two methods were employed using the modified method of Limonard (1966) and Tempe (1967). (i) A single flower with its stigmatic surface was placed on sterile Whatman No.1 filter paper in a 9-cm diameter sterile Petri dish moistened with 5-10 ml of sterile distilled water. Thirty replicates (10 from each population) were used. (ii) A single flower with its stigmatic surface was placed on three solid media (PDA, DG-18, and OGYE), for each population (total sample = 30). In both methods, samples were incubated at 28-32°C in each instance (i and ii) until fungi grew (after 3 days). The number of colonies per flower was recorded. The fungi which appeared were identified using their cultural, colour and morphological characteristics. In the case of flowers placed on the filter paper only, the species were observed and identified using a binocular stereomicroscope (Leica Zoom, 2000 model, Germany). Both flowers and isolated stigmata were studied (data in tables come only from the latter).

### Data analysis

All statistical analyses were performed on MINITAB version 15. Analysis of variance (ANOVA) was performed to compare the distance from *Talbotiella* populations on fungal infestation and also test for normality of the data before used for analysis. A *t*-test was used to discriminate between the means at $P < 0.05$.

## RESULTS

The highest mean count of air-borne fungal colonies (389 ± 2.1 cfu/p) from three populations was recorded for Yongwa with 20 min exposure on PDA followed by Sapawsu. Consistent differences among sites, at all exposure times were recorded (**Table 1**). A total of 26 fungal species in 17 genera was recorded. The genera *Aspergillus*, *Penicillium* and *Cladosporium* recorded highest number of colonies. *Penicillium aurantiogriseum* and *Fusarium verticilloides* were the dominant species within *Talbotiella* populations (**Table 2**). Isolation of fungi from stigmatic surfaces revealed *Fusarium verticilloides* as dominant species forming 91.74% of the total fungi isolated from stigmatic surfaces of *Talbotiella* (**Table 3**). ANOVA showed no significant effect ($P$ = 0.08) of distance from *Talbotiella* population on the total number of fungal colonies isolated on the three media. Most

**Table 1** Total number of colonies of aeromycoflora isolated by the Plate exposure method at the indicated exposure intervals within populations of *Talbotiella* at Yongwa, Sapawsu and Hotel in 2006 (N = 60, ± s.e.).

| Site | Medium | Mean no. of colonies isolated per plate after (min) exposure | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 |
| Yongwa | PDA | 50 ± 0.2 | 58 ± 0.3 | 194 ± 1.1 | 389 ± 2.1 |
| | OGYE | 18 ± 0.4 | 84 ± 0.5 | 91 ± 2.8 | 108 ± 2.3 |
| | DG18 | 26 ± 0.2 | 64 ± 0.7 | 98 ± 1.9 | 221 ± 2.6 |
| | Total | 94 | 206 | 383 | 718 |
| Sapawsu | PDA | 22 ± 1.2 | 49 ± 0.9 | 192 ± 2.1 | 309 ± 3.2 |
| | OGYE | 39 ± 2.3 | 47 ± 0.5 | 62 ± 1.6 | 89 ± 2.3 |
| | DG18 | 35 ± 0.9 | 54 ± 1.0 | 132 ± 0.6 | 257 ± 109 |
| | Total | 96 | 150 | 386 | 655 |
| Hotel | PDA | 17 ± 0.7 | 28 ± 1.7 | 173 ± 2.3 | 261 ± 2.8 |
| | OGYE | 27 ± 1.5 | 50 ± 1.2 | 75 ± 1.4 | 101 ± 1.3 |
| | DG18 | 21 ± 0.8 | 53 ± 2.2 | 168 ± 4.1 | 227 ± 1.2 |
| | Total | 65 | 131 | 416 | 589 |

**Table 2** Mean number of colonies per plate (N=30) of individual fungal species isolated by three media (data pooled) at different exposure times within three populations (Yongwa, Sapawsu and Hotel) of *Talbotiella* in 2006.

| Fungi recorded | Mean number (Mean ± SE) of individual species at the three populations studied for (min) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yongwa | | | | Sapawsu | | | | Hotel | | | |
| *Aspergillus alutaceus* | **5** | **10** | **15** | **20** | **5** | **10** | **15** | **20** | **5** | **10** | **15** | **20** |
| *A. flavus* | 1.8 ± 0.5 | 4.4 ± 0.9 | 7.1 ± 0.3 | 8.9 ± 0.2 | 4.1 ± 0.6 | 8.2 ± 0.7 | 9.4 ± 06 | 10.2 ±.4 | 1.2 ± 0.6 | 3.2 ± 0.5 | 4.1 ± 0.9 | 5.3 ±.13 |
| *A. niger* | 3.9 ± 0.3 | 5.3 ± 0.4 | 8.4 ± 1.7 | 10.2 ± 1.2 | - | - | - | - | 0.8 ± 0.7 | 1.1 ± 0.9 | 2.8 ± 0.9 | 3.1 ± 0.4 |
| *A. sulphurous* | 2.2 ± 0.2 | 3.1 ± 0.2 | 3.7 ± 0.3 | 4.9 ± 0.5 | 0.4 ± 0.2 | 0.7 ± 0.1 | 0.9 ± 0.2 | 2.4 ± 0.6 | 0.35 ± 0.5 | 1.2 ± 0.4 | 1.3 ± 0.4 | 2.6 ± 0.3 |
| *Alternaria sp.* | 0.3 ± 0.1 | 0.5 ± 0.2 | 1.8 ± 0.1 | 4.1 ± 0.2 | - | - | - | - | - | - | - | - |
| *Absidia cylindrospora* | 6.3 ± 0.2 | 7.9 ± 1.2 | 11.5 ± 0.9 | 14.1 ± 0.3 | 0.2 ± 0.6 | 0.4 ± 0.1 | 1.8 ± 4.1 | 4.1 ± 0.3 | 1.2 ± 0.3 | 3.2 ± 0.5 | 4.1 ± 0.9 | 5.4 ± 0.2 |
| *Cladosporium herbarum* | 2.2 ± 1.7 | 4.2 ± 1.5 | 6.9 ± 0.8 | 12.8 ± 0.6 | - | - | - | - | - | - | - | - |
| *C. cladosporioides* | 11.5 ± 0.2 | 15.5 ± 0.7 | 19.2 ± 0.5 | 21.4 ± 0.6 | 9.3 ± 2.3 | 14.8 ± 1.9 | 21.7 ± 1.6 | 24.8 ± 1.2 | 4.5 ± 1.2 | 6.1 ± 1.6 | 7.9 ± 1.1 | 11.6 ± 0.7 |
| *C. macrocarpum* | 0.3 ± 0.2 | 3.8 ± 1.4 | 5.6 ± 2.1 | 6.3 ± 0.9 | 0.4 ± 0.9 | 6.2 ± 0.3 | 6.6 ± 1.3 | 7.3 ± 1.2 | - | - | - | - |
| *Fusarium verticilloides* | 5.7 ± 0.5 | 6.2 ± 0.2 | 7.4 ± 0.4 | 13.8 ± 0.8 | 4.9 ± 0.9 | 8.4 ± 0.6 | 15.1 ± 0.9 | 20.4 ± 1.2 | 0.6 ± 0.5 | 0.7 ± 0.1 | 3.4 ± 0.5 | 9.8 ± 0.9 |
| *Helminthosporium sp.* | 10.3 ± 2.3 | 33.4 ± 1.2 | 39.5 ± 0.3 | 41.1 ± 1.8 | 25.2 ± 1.8 | 37.8 ± 1.4 | 43.4 ± 1.6 | 55.8 ± 3.4 | 17.9 ± 1.9 | 54.8 ± 3.6 | 64.3 ± 2.4 | 67.4 ± 2.8 |
| *Mucor haemalis* | 1.4 ± 0.7 | 1.9 ± 0.9 | 2.1 ± 1.6 | 1.2 ± 0.3 | - | - | - | - | 0.4 ± 0.1 | 1.6 ± 1.3 | 1.7 ± 1.1 | 0.9 ± 0.8 |
| *Mycelia sterilia* | 0.7 ± 0.5 | 1.3 ± 0.8 | 2.6 ± 0.3 | 3.2 ± 0.2 | - | - | - | - | 1.4 ± 2.3 | 2.2 ± 0.4 | 2.7 ± 0.3 | 4.2 ± 0.7 |
| *Penicillium aurantiogriseum* | - | - | - | - | - | - | - | - | 6.1 ± 0.2 | 6.4 ± 0.3 | 7.3 ± 0.5 | 14.8 ± 0.2 |
| *P. camemberti* | 13.2 ± 0.7 | 39.4 ± 1.2 | 44.6 ± 1.8 | 51.7 ± 1.8 | 30.1 ± 1.8 | 54.6 ± 1.6 | 59.4 ± 2.3 | 61.2 ± 1.8 | 8.1 ± 0.2 | 12.3 ± 1.3 | 17.2 ± 0.9 | 24.6 ± 1.3 |
| *P. expansum* | 3.2 ± 03 | 3.9 ± 1.6 | 6.7 ± 0.6 | 7.5 ± 0.2 | 1.4 ± 1.6 | 5.5 ± 0.8 | 7.3 ± 0.3 | 9.1 ± 0.1 | 0.9 ± 0.1 | 1.2 ± 0.4 | 1.6 ± 0.7 | 2.1 ± 0.1 |
| *P. glabrum* | 13.1 ± 2.1 | 28.4 ± 2.2 | 45.5 ± 2.5 | 61.1 ± 1.1 | 23.0 ± 1.1 | 48.3 ± 09 | 55.5 ± 1.5 | 58.3 ± 1.3 | 12.6 ± 0.6 | 16.3 ± 0.4 | 19.2 ± 0.3 | 28.2 ± 1.4 |
| *Paecilomyces sp.* | 21.9 ± 0.8 | 34.7 ± 2.5 | 43.9 ± 1.5 | 52.1 ± 1.6 | 13.9 ± 1.4 | 19.4 ± 1.3 | 34.6 ± 0.8 | 41.2 ± 1.1 | 8.6 ± 1.3 | 11.4 ± 0.9 | 17.3 ± 2.1 | 39.9 ± 1.7 |
| *Pichia sp.* | - | - | - | - | 4.9 ± 0.4 | 8.6 ± 0.5 | 13.4 ± 0.6 | 19.2 ± 0.7 | - | - | - | - |
| *Pullularia pullulans* | - | - | - | - | 10.2 ± 2.5 | 15.4 ± 1.2 | 19.8 ± 0.9 | 31.2 ± 9.8 | 8.3 ± 1.7 | 12.4 ± 0.9 | 18.3 ± 1.3 | 23.4 ± 1.4 |
| *Rhodotorula sp.* | 9.8 ± 1.4 | 12.3 ± 1.6 | 15.3 ± 1.3 | 21.7 ± 0.9 | 11.3 ± 0.8 | 12.4 ± 2.3 | 18.3 ± 0.6 | 8.9 ± 0.4 | 0.6 ± 0.4 | 9.2 ± 0.2 | 11.6 ± 0.6 | 13.1 ± 0.8 |
| *Scopulariopsis brevicaulis* | 0.3 ± 0.2 | 0.7 ± 0.4 | 3.6 ± 0.6 | 8.9 ± 1.4 | - | - | - | - | - | - | - | - |
| *S. fusca* | - | - | - | - | 4.3 ± 1.2 | 9.7 ± 0.3 | 14.2 ± 1.6 | 17.9 ± 0.9 | 3.9 ± 0.5 | 5.7 ± 0.7 | 6.6 ± 1.1 | 8.9 ± 0.7 |
| *Syncephalastrum racemosum* | 10.8 ± 0.9 | 11.3 ± 1.6 | 12.3 ± 1.3 | 12.9 ± 1.3 | 6.8 ± 1.4 | 7.1 ± 1.6 | 7.8 ± 0.3 | 8.7 ± 1.5 | - | - | - | - |
| *Trichoderma viride* | 1.2 ± 0.4 | 2.7 ± 0.2 | 3.6 ± 0.6 | 5.9 ± 1.7 | - | - | - | - | - | - | - | - |
| *Yeast indet* | - | - | - | - | 0.9 ± 0.4 | 1.4 ± 0.3 | 1.6 ± 0.2 | 1.8 ± 0.3 | - | - | - | - |
| Total colonies | 4.3 ± 1.2 | 5.7 ± 0.4 | 6.6 ± 0.6 | 7.8 ± 1.1 | - | - | - | - | 1.9 ± 0.2 | 2.7 ± 0.4 | 2.9 ± 0.6 | 3.1 ± 10.8 |
| | 124.4 | 226.6 | 297.9 | 371.6 | 151.3 | 258.9 | 330.8 | 345.4 | 79.35 | 151.7 | 194.3 | 268.4 |

**Table 3** Isolation of contaminating fungi on stigmatic surface of flower of *Talbotiella* at three populations (Yongwa, Sapawsu and Hotel) in 2006. Infection was measured 2-6 days after flower opening to expose the stigma.

| Site | Days after flower opening | Total no. of colonies | % occurrence of *F. verticilloides* |
|---|---|---|---|
| Yongwa | 2 | 5 | 100 |
| | 4 | 9 | 100 |
| | 5 | 12 | 91.7 |
| | 6 | 15 | 86.7 |
| Sapawsu | 2 | 2 | 100 |
| | 4 | 7 | 100 |
| | 5 | 14 | 85.7 |
| | 6 | 19 | 73.7 |
| Hotel | 2 | 4 | 100 |
| | 4 | 6 | 100 |
| | 5 | 11 | 81.8 |
| | 6 | 16 | 81.3 |

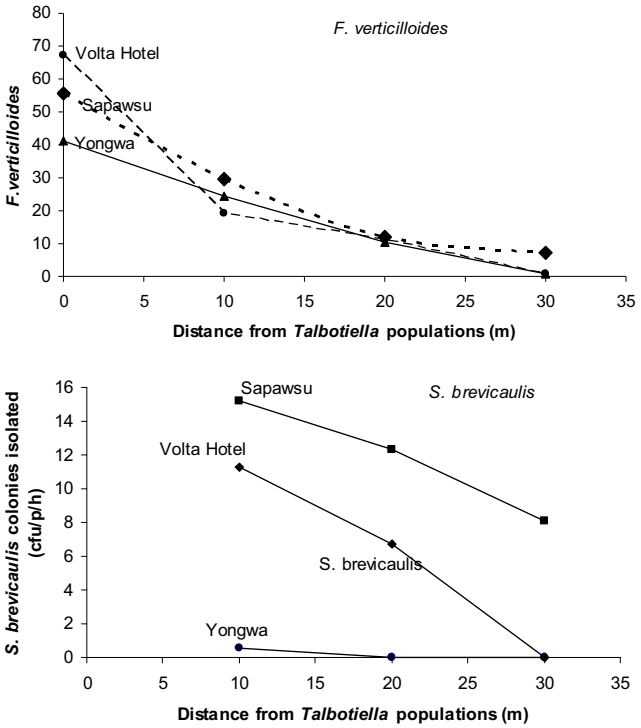


**Fig. 2 Effect of distance from *Talbotiella* populations (Yongwa, Sapawsu and Hotel) on *Fusarium verticilloides* and *Scopuriopsis brevicaulis* spores in air after 20 min of exposure time of media (pooled results).**

species had no clear relationship with distance, except *F. verticilloides* and *S. brevicaulis* that decreased with distance from *Talbotiella* populations (**Fig. 2**). Even though total fungal spore concentration in air was not significantly affected by distance from *Talbotiella* populations ($P$ = 0.08), concentration of *Fusarium verticilloides* (**Fig. 3E-G**) and *Scopulariopsis brevicaulis* in air decreased significantly with distance from *Talbotiella* populations ($P$ = 0.000). Fungi were found on all parts of flowers of *Talbotiella* (**Figs. 3H-L**). Style and ovary were infested by several species in addition to *Fusarium verticilloides* but the stigmatic surface was mainly infested with *F. verticilloides* (**Figs. 3E, 3F**).

## DISCUSSION

The open Petri-dish, nutrient plate, plate exposure or sedimentation method have been widely used by investigators (Gravesen 1978; Snealler 1979; Sen and Asan 2001; Sen *et al.* 2010; Yassin and Almouqatea 2010) to isolate air-borne fungal spores due to their practical usage and low cost. However, these methods give only a rough approximation of the counts (Pelczar *et al.* 1993) and their reliability could be affected by the size and shape of the spore and air turbulence. Very small spores might never settle therefore the plate was left out longer in order to counteract the air turbulence effect. Though the concentration of fungal spores in air in *Talbotiella* stands differed from one site to another,

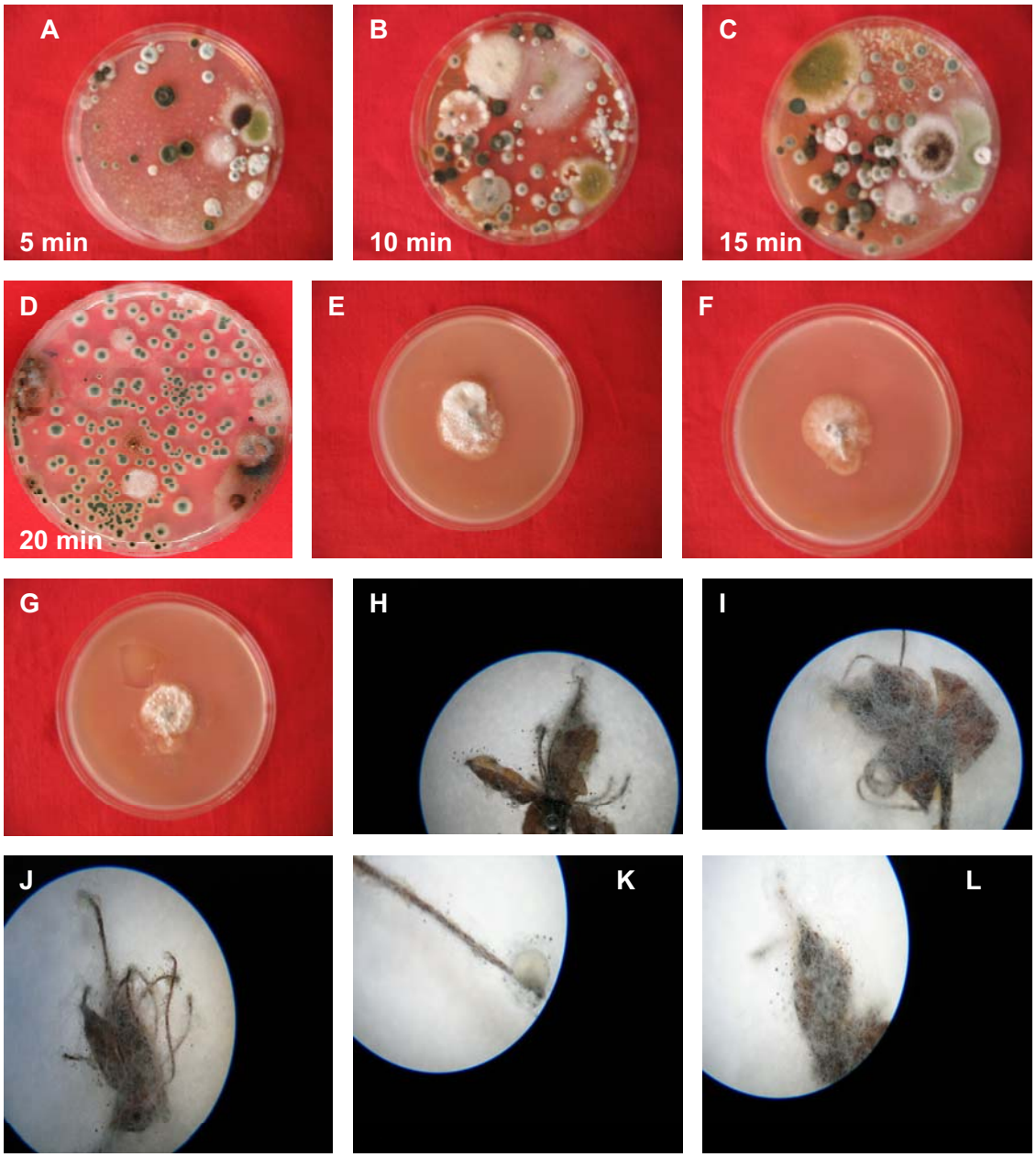


**Fig. 3** Fungal colonies (**A**) 5 min, (**B**) 10 min, (**C**) 15 min, (**D**) 20 min exposure time on PDA agar medium; (**E**) *F. verticilloides* isolates from stigmatic surface from Yongwa, (**F**) from Sapawsu and (**G**) from Hotel on PDA agar medium. (**H**) fungal growth on flower of *Talbotiella* from Yongwa, (**I**) Sapawsu and (**J**) Hotel on Whatman's no. 1 filter paper, (**K**) fungal growth on stigma and style and (**L**) on the ovary of *Talbotiella* on Whatman's no. 1 filter paper.

there was no clear difference in the composition of species isolated on the three media from the three populations studied. *Penicillium aurantiogriseum* and *Fusarium verticalloides* were the most frequently encountered fungi in the three populations studied (**Table 2**). In the literature, *Aspergillus* and *Penicillium* species have been reported as common fungal genera all over the world (Pasanen *et al*. 1993) and *Penicillium* has also been found to dominate most regions in terms of numbers (Rosa *et al.* 1988). Pepeljnjak and Šegvić (2003) similarly reported high numbers of *Cladosporium*, *Penicillium* and *Alternaria* in the air of all three climatic regions in Croatia, while *Fusarium* was the most prevalent fungal genera on plant samples especially in the modest continental climate.

The high numbers of *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium* and *Pullaria* species recorded in all the populations studied may be attributed to their ability to grow in various substrata and weather conditions. Though weather conditions were not taken into consideration in this study, *Aspergillus* species have been found to dominate humid environments (Lacey 1991) therefore investigation into the influence of weather conditions on fungal densities in *Talbotiella* populations may be important. Di-Giorgio *et al*. (1996) and Oliveira (2008) found that various meteorological factors affect type and concentration of air-borne fungi. Corden and Millington (2001), Awad (2005) found a positive correlation between *Alternaria* counts and maximum temperature. Falling raindrops wash out solid materials including airspora which leads to cleaner air (Cariñanos *et al.* 2001).

The highest record of fungal colonies was recorded on PDA (389 ± 3.2 cfu/p) and DG18 (227 ± 1.2 cfu/p), thus confirming the usefulness of these two media (**Table 1**). OGYE supported least growth of fungal species belonging to eight genera. None of the three media was selective for different fungal groups.

The deposition of fungal spores on stigmatic surfaces of *Talbotiella* may affect pollen performance in two ways (1) physical interference (2) chemical interference. In physical interference, the stigmatic surface area available for pollen germination is reduced. Pollen therefore competes for germinating space. In chemical interference, pollen is

weakly bound to the stigmatic surface and tube growth is also chemically inhibited. According to Bedinger (1992) and Heslop-Harrison (1992), in order for pollen to be successful, the pollen grain must bind to the stigmatic surface, take up water, germinate and grow down the stylar tissue. However, fungal spores also need water to germinate and conjugate (Fisher and Holton 1957; Ruddat and Kokontis 1988; Day and Garber 1988) which could interfere with the binding of pollen to the stigmatic surface. Spores interfering with pollen may lead to dehydration and germination failure of pollen.

Many fungi produce enzymes that degrade cell walls to aid in penetrating host tissue (Cooper 1983; Keon *et al*. 1987). Some fungal metabolites are also toxic to plant cells if present in sufficient concentrations (Kono *et al.* 1981). Compatible fungal mating types may fuse upon landing on the stigmatic surface resulting in growth of hyphae through the flower into the plant and may affect ovules or seed development if fertilization has occurred. This may explain low seed set in *Talbotiella*.

Similar work by Thomas *et al.* (1994) on avocado trees and Marr (1998) on *Silene acaulis* suggested that floral pathogens may interfere with pollen function. They observed poor fruit set in Avocado trees, even during seasons when conditions were favourable for fertilization and fruit production where about 80% of the styles from open pollinated flowers were colonized by a variety of fungi associated with about 90% of flower abortion.

In *Talbotiella*, *Fusarium verticilloides* appeared to be the main fungus interfering with pollen germination (**Table 3, Fig. 3**) but it is not known whether this species produced toxins on stigmatic surface to inhibit pollen germination. *Fusarium verticilloides* produces fusaric acid (5-butylpicolinic acid) which is a mycotoxin (Stoll *et al.* 1957; Vesonder *et al.* 1981; Mutert *et al.* 1990) and could inhibit pollen germination on the stigmatic surface. Another mycotoxin known to be produced by *Fusarium verticilloides* is moniliformin (Kriek *et al.* 1977; Steyn 1978). *Fusarium verticalloides* has been described as a species complex composed of six different biological species, frequently termed mating populations (Leslie 1991, 1995).

Some of the populations of *Talbotiella* are located near farming communities and some of the isolated fungal spores may be associated with agricultural practices. According to Booth (1971), *Fusarium verticilloides* and *Fusarium sacchari*, are common maize pathogens in most maize producing areas of the world. Marasas *et al*. (1979) found that both species occur commonly in maize seed in South Africa. Sreeramulu and Ramalingam (1961) also noted that crop harvest coincides with increased concentration of fungi particularly *Alternaria* species. Though one of the main agents of removing spores from exposed spore mass into the air is wind velocity (Sreeramulu 1961; Aylor 1986, 2003), spores are also transmitted by pollinators that visit diseased plants and transport spores to healthy plants (Baker 1947; Roy 1994; Marr 1997). As Jennersten (1988), Alexander (1990), Roche *et al.* (1995), Shykoff and Bucheli (1995) and Cunningham (2000) fungal spores are transmitted by pollinators, and floral infection is the most common mode of disease transmission. A majority of flowers receive pollen and spores together during pollination therefore pollen and spores may interact and may be one of the modes of transmission of diseases in *Talbotiella* populations. Studies on *Silene latifolia*, *S. dioica* and *S. acaulis* have shown that seed set is lower in healthy flowers receiving both fungal spores and pollen together compared with flowers receiving only pollen (Alexander 1987; Carlsson 1995; Marr 1997; Curran *et al*. 2009).

Concentrations of *Fusarium verticilloides* and *Scopulariopsis brevicaulis* in air decreasing significantly with distance from *Talbotiella* populations suggests that *Fusarium verticilloides* are associated with *Talbotiella* but the type of association may requires further investigation. Populations of *Talbotiella* are infested with spores of fungi belonging to 17 genera. The spores infect all parts of flowers of *Talbotiella* but the stigmatic surface was mainly infested with *Fusarium verticilloides. Fusarium verticalloides* may physically and chemically affect pollen performance on stigmatic surface and may also interfere with normal fertilization and seed development. They may also be contributing to fruit abortion in *Talbotiella*.

## Conservation implications

Though populations of *Talbotiella* are infested with many fungal spores, the alarming rate of infestation of the stigmatic surface by *Fusarium verticilloides* requires further investigation to determine the potential pathogenic impact on pollination and fruit set in *Talbotiella* in order to apply better conservation strategies to prevent *Talbotiella* from extinction.

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