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Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*

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Abstract

Splash dispersal of *Fusarium culmorum* and *Fusarium poae* spores was studied, using inoculated straw placed on tiles as the inoculum source to infect agar strips and artificially produced leaves. In addition, patterns of spread were studied with spores from inoculated artificial leaves onto agar strips. Observed patterns of spore dispersal for each species were indistinguishable, although *F. culmorum* produced fewer colonies than *F. poae*. Furthermore, spore dispersal from inoculated straw and artificial leaves were essentially identical, with one exception; colonies arose from single conidia when spread from artificial leaves, but consisted of clumps of conidia when derived from inoculated straw. Splash dispersal patterns of both species onto the upper- and undersides of artificial leaves were different. On the upperside of the leaf, most colonies were found at the tip, while on the underside of the leaf most colonies were found at the base of the leaf. This is the first time that artificially produced leaves have been used in splash dispersal experiments.

Introduction

Fusarium spp. are important pathogens of commercial crops, such as wheat, maize and rice. These fungi are capable of producing toxins, affecting both human and animal health (Mills, 1989). To be able to control these pathogens, knowledge about their dispersal patterns is essential. After wind dispersal, the second most important factor for fungal disease dissemination is splash dispersal (Madden, 1992). A previous study indicated that rain is the most important factor for dispersal of *Fusarium* spp. (Rossi et al., 2000).

In general, raindrops reaching the ground are restricted to a diameter of 0.2–5.0 mm, since larger drops are likely to break up and smaller drops would normally evaporate (Madden, 1992). When hitting the ground, each drop disperses into droplets, which travel distances indirectly proportional to their size. Moreover, when the drops hit spore-containing surfaces, the spores are incorporated into the droplets (Fitt and Lysandrou, 1984). In disease dissemination, splash dispersal caused by larger droplets is considered of greater importance as they tend to incorporate more spores (Brennan et al., 1985; Fitt and Lysandrou, 1984; Fitt et al., 1988). It has also been shown that a plain surface can extensively spread more spores compared to a rough surface, e.g. plant debris (Fitt et al., 1989; Madden, 1997; Walklate et al., 1989). In earlier trials, when splash height was studied, it was noted that the maximum splash height reached was higher for straw supported on a plain surface than in the absence of straw. The splash height was further reduced in the presence of unsupported straw (Walklate et al., 1989). Thus, the use of straw supported on plain surfaces would provide a useful model to study the effects of splash dispersal, mimicking the combined effect of straw and accumulated water that commonly occurs in the field during rainfall. This model system is particularly appropriate for the study of fungal pathogens like *Fusarium*, with spores

contained in mucilage that may require some wetting to necessitate their release (Fitt and McCartney, 1986).

Previous studies revealed that fungi disseminated by splash dispersal show steep dispersal gradients, in which the number of spores decreases in proportion to the height and distance from the original source (Brennan et al., 1985; Fitt and Lysandrou, 1984; Fitt et al., 1988; Jenkinson and Parry, 1994; Pedersen et al., 1994). While the mechanisms of splash dispersal have been studied for numerous pathogens, studies of *Fusarium* dispersal have been restricted to a still-air experiment and a field experiment, investigating *F. culmorum* (W.G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc., *F. graminearum* Schwabe and *Microdochium nivale* (Fr.) Samuels and Hallett (Jenkinson and Parry, 1994; Rossi et al., 2000). These authors showed that rain plays a very important role for dispersal of *Fusarium* conidia. Also the dispersal patterns of *F. avenaceum* and *F. culmorum* resembled each other (Jenkinson and Parry, 1994). There are several records of *F. culmorum* being retrieved from stem bases and nodes, while *F. poae* has only been found at very low frequencies and the records are only from stem bases (Polley and Turner, 1995; Bateman et al., 1998; Hall and Cutton, 1998). Both species have been noted in the head blight complex (Polley and Turner, 1995; Doohan et al., 1999).

This study reports the first findings concerning the patterns of splash dispersal of *F. poae* (Peck) Wollenw, which mainly produces small, round microconidia. For comparison, *F. culmorum*, which produces falcate, slightly curved macroconidia was included, since the splash dispersal pattern by this pathogen has been documented (Jenkinson and Parry, 1994). Because of the different shapes of the spores, it was of interest to see whether the dispersal patterns differed between these two pathogens. Furthermore, a novel method for the study of splash dispersal patterns onto artificially produced leaves, constructed from transparency film covered with a thin film of agar under controlled conditions, is reported. The incorporation of artificial leaves in splash dispersal studies is of significance for improved understanding of how the pathogen is spread onto the plant, since this provides a system that allows the study of spread onto leaves with a low risk of contamination, and without interference from the plant. Finally, it was of interest to see whether the inoculum arrived on the leaf close to the stem or at the tip of the leaf.

Materials and methods

Preparation of inoculated straw and production of water droplets

The fungal isolates used were *F. poae* IBT 1514 and *F. culmorum* IBT 1512, both of which were stored on soil at $+8\,^{\circ}$C. Fungal inoculum was prepared according to Jenkinson and Parry (1994), with the exception that straws incubated for more than 14 days were stored at $+8\,^{\circ}$C to suppress mycelial growth. To produce water drops of 4.5 mm diameter, a burette was used. The diameter of the drops was calculated according to Jenkinson and Parry (1994).

Splash dispersal onto agar strips

Splash dispersal onto agar strips was performed using a modification of the method of Jenkinson and Parry (1994). Plastic moulds were constructed by milling a 3 cm wide and 0.4 cm deep groove in a 106 $\times$ 5 $\times$ 0.5 cm piece of polymetylmetakrylat. Specialised nutrient poor agar medium (Spezieller Nahrstoffarmer Agar, SNA) (Nirenberg, 1976) was poured to a depth of 2–3 mm into 70% ethanol washed moulds.

Each 100 cm long agar strip was placed in a vertical position in which the bottom end was 3 cm from ground level. Ten agar strips were placed in a spiral arrangement, according to Jenkinson and Parry (1994), at distances ranging from 10 to 100 cm from the source of inoculum, onto which 50 drops of sterile water were released. To avoid overloading, the strip closest to the source (i.e. at 10 cm distance) was removed after 10 drops. The experiment was repeated three times for each species. The number of colonies dispersed onto each agar strip was assessed by light microscopy ($\times$50) following 48 h of incubation. Only viable colonies were counted.

Splash dispersal onto artificial leaves

Artificial leaves were constructed from transparency film, normally used in standard plain paper copiers (PP 2500, 3M Visual Systems Products, France). The leaves measured 0.7 cm at the base, 0.2 cm at the tip and 1.4 cm at the broadest part located 7.2 cm from the base. Each leaf was 21 cm in length. To attach each leaf, a triangular shape of 1.4 (base) $\times$ 0.6 (tip) $\times$ 3 (length) cm was included, arranged such that the point of the triangle was located towards the basal part of the leaf.

All leaves were pre-sterilised with 70% EtOH and then sprayed with a thin film of SNA on the upper surface for use in one trial, and on the under surface for use in a second trial. To stabilise the leaves and make them more authentic, a thin metal thread (0.8 mm × 5 cm) was attached with tape to the side lacking agar. The leaves were attached to two wooden poles, three leaves on each pole, so that the leaf base was placed at 10, 20 and 30 cm from ground level, and the tips were at a distance of 1, 11 and 21 cm from the ground, respectively. The poles were placed 20 and 30 cm from the source of inoculum, with the leaf tips facing the inoculum, composed of *F. culmorum* and *F. poae* inoculated on straw placed on tiles (Jenkinson and Parry, 1994). To initiate splash dispersal, 30 drops of sterile water, 4.5 mm in diameter, were released from a height of 6 m. The drops reached terminal velocity before hitting the inoculum (Jenkinson and Parry, 1994). All experiments were performed in triplicate for each species and for each side of the leaf.

The artificial leaves were placed agar-side facing up, into sealed plastic chambers containing drops of sterile water and incubated for 48 h at room temperature. Each leaf was raised from the base of the moistened chamber using four Petri dishes as solid support. Colony numbers were assessed by dividing the leaves into four equally sized areas and viewing them by light microscopy (×50).

Artificial leaves as source of inoculum

From each individual splash dispersal experiment with artificial leaves, the most infected leaf was placed in moist chambers for one week to allow sporulation. After incubation the leaves were carefully taped back onto the wooden poles as in the previous experiment, but this time only 10 cm above ground level. The burette was placed 6 m above the leaf and 50 drops of 4.5 cm diameter were released onto the leaf. Two agar strips were placed at a distance of 10 and 20 cm from each side of the leaf. After the first 10 drops, the agar strip 10 cm from the source was removed to avoid overloading. The experiment was performed in triplicate for each species. The agar strips were then treated according to Jenkinson and Parry (1994).

Statistical analysis

Effects of height, distances, interaction between height and distance, sides of the leaf and parts of the leaf were analysed by using the program SYSTAT version 5.2.1 (SYSTAT inc.) for a parametric analysis of variance. Horizontal and vertical deposition gradients were estimated by Poisson regression and values for half-distances (d_{50}) and half-heights (h_{50}) were calculated using the Statistical Analysis System (SAS Insitute Inc., Cary, NC 27513, USA).

Results

In all cases, microscopic examination of both agar strips and artificial leaves showed that all colonies arose from germinating conidia. All conidia that had reached the agar were viable.

Splash dispersal onto agar strips

Infected straw as source of inoculum

When infected straw was used as the source of inoculum, both *F. poae* and *F. culmorum* displayed indistinguishable splash dispersal properties. In particular, there was a significant correlation between vertical height and horizontal distance to which the spores travelled. Moreover, both pathogens displayed a steep gradient in the number of colonies, such that highest numbers were recorded close to the source and subsequently declined with increased distance (Figures 1 and 2). *F. poae* colonies were found at a maximum vertical height of 58 cm and a maximum horizontal distance of 70 cm (Figure 1). More than half the colonies for *F. poae* were found at a vertical height of 4–15 cm (Table 1) and a horizontal distance of 8–15 cm (Table 2). The colonies of *F. culmorum* colonies were found at a maximum vertical height of 58 cm and a maximum horizontal distance of 100 cm (Figure 2). The half-distances for this pathogen were 4–13 cm in vertical height (Table 3) and 7–13 cm in horizontal distance (Table 4), similar to *F. poae*. However, a significant difference ($P < 0.0005$) in the number of colonies recorded for each pathogen was observed. The maximum number of colonies observed for *F. poae* was close to 3000, while only 500 were recovered for *F. culmorum* (Figures 1 and 2). In addition, *F. poae* colonies always consisted of clumps of several hundred spores. On the other hand, some *F. culmorum* colonies did arise from single conidia, although most colonies did contain spore masses. The number of spores within each colony was always lower for *F. culmorum* compared to *F. poae*.

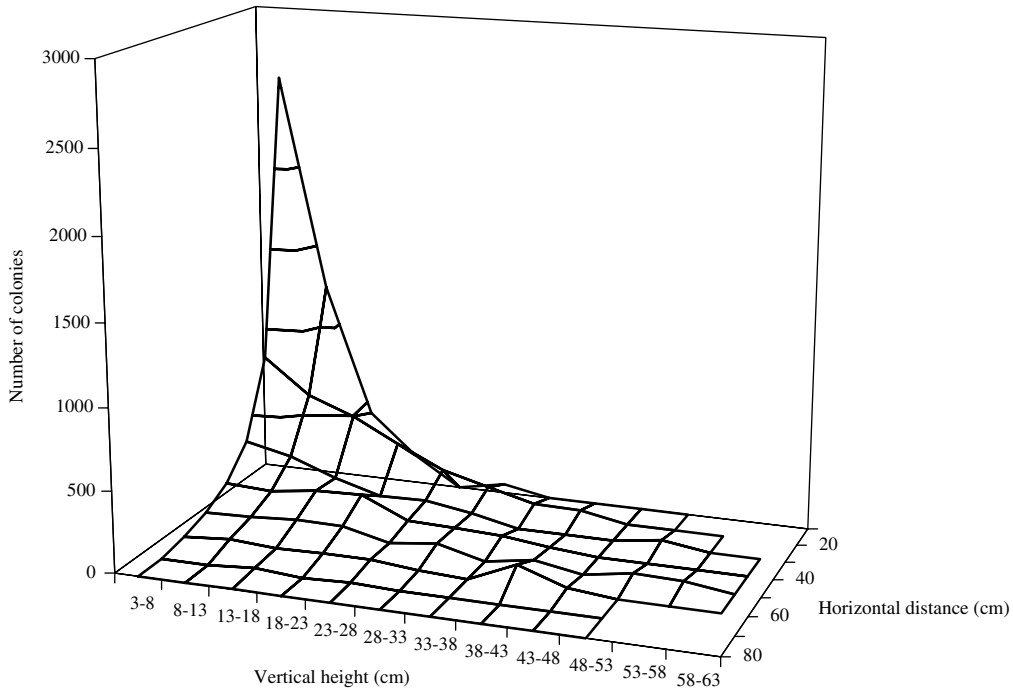

Figure 1. Mean number of *F. poae* colonies on agar strips at various vertical heights and horizontal distances from the original inoculum source, following splash dispersal of spores by waterdrops released from a height of 6 m.

Table 1. Vertical deposition gradients for conidia of *F. poae* dispersed by simulated raindrops, 4.5 mm in diameter, from infected straw. Slopes (b) and half-distances (d_{50}) estimated by Poisson regression

Horizontal distance from source (cm)	Vertical b	Deposition h_{50} (cm)
10	0.183	3.8
20	0.089	7.8
30	0.087	8.0
40	0.068	10.2
50	0.047	14.7

Table 2. Horizontal deposition gradients for conidia of *F. poae* dispersed by simulated raindrops, 4.5 mm in diameter, from infected straw. Slopes (b) and half-distances (d_{50}) estimated by Poisson regression

Vertical height above source (cm)	Horizontal b	Deposition d_{50} (cm)
3–8	0.090	7.7
8–13	0.073	9.5
13–18	0.056	12.4
18–23	0.050	13.9
23–28	0.047	14.7

Artificial leaves as a source of inoculum

In a further effort to simulate splash dispersal in the field, artificial leaves were designed, using transparency film. These artificial leaves were used as the source of the inoculum in the following experiments. There was no significant difference ($P = 0.5786$) between the splash dispersal patterns of either pathogen using artificial leaves as the inoculum source compared to inoculated straw placed on tiles. In particular, the number of colonies and the distances to which they spread were indistinguishable regardless of the pathogen and source of inoculum (data not shown). However, one notable difference was that most colonies arose from single spores. This was in contrast to earlier observations.

Splash dispersal onto artificial leaves

Both pathogens showed a similar splash dispersal pattern. However, the dispersal patterns on the under- and uppersides of the leaves were significantly different (*F. culmorum* $P = 0.14$ and *F. poae* $P = 0.007$).

On the underside of the leaf, most colonies were found at the base of the leaf, decreasing towards the tip

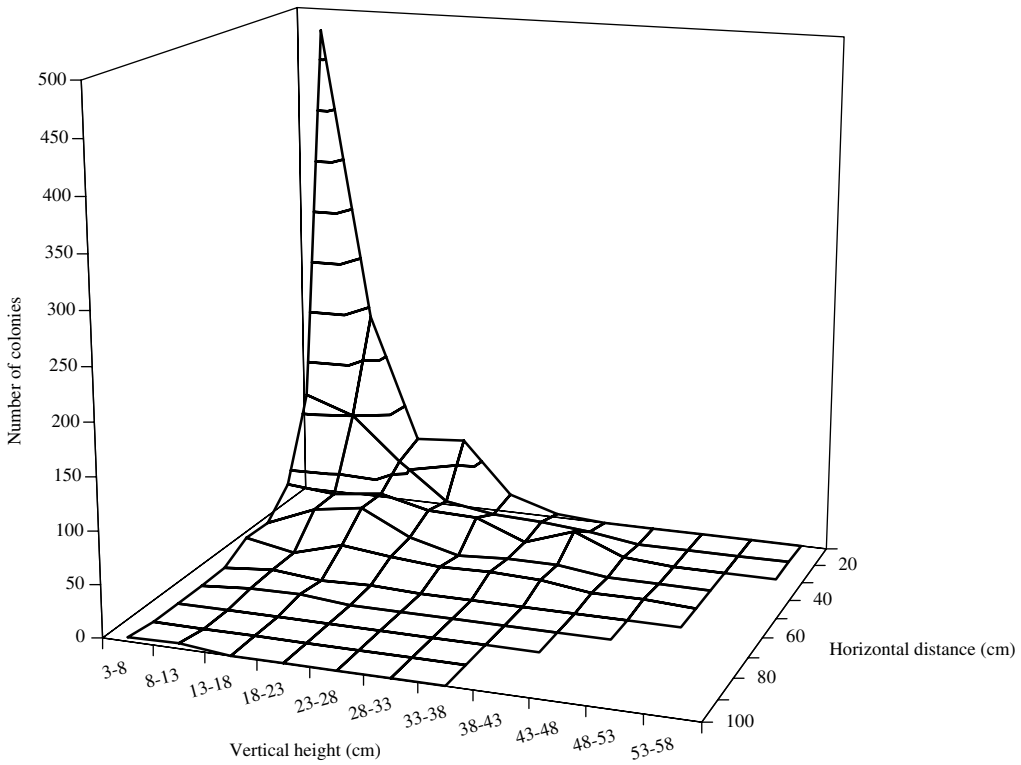

Figure 2. Mean number of *F. culmorum* colonies on agar strips at various vertical heights and horizontal distances from the original inoculum source, following splash dispersal of spores by waterdrops released from a height of 6 m.

Table 3. Vertical deposition gradients for conidia of *F. culmorum* dispersed by simulated raindrops, 4.5 mm in diameter, from infected straw. Slopes (b) and half-distances (d_{50}) estimated by Poisson regression

Horizontal distance from source (cm)	Vertical b	Deposition h_{50} (cm)
10	0.169	4.1
20	0.110	6.3
30	0.065	10.7
40	0.082	8.5
50	0.055	12.6

Table 4. Horizontal deposition gradients for conidia of *F. culmorum* dispersed by simulated raindrops, 4.5 mm in diameter, from infected straw. Slopes (b) and half-distances (d_{50}) estimated by Poisson regression

Vertical height above source (cm)	Horizontal b	Deposition d_{50} (cm)
3–8	0.099	7.0
8–13	0.067	10.3
13–18	0.052	13.3
18–23	0.055	12.6
23–28	0.054	12.8

(Figures 3 and 4). In contrast, most colonies on the upperside of the leaf were found at the tip, decreasing towards the base (Figures 3 and 4). Moreover, leaves nearest to the ground level exhibited the highest colony density, while the leaves at 30 cm were virtually colony free (data not shown). On central leaves (20 cm from ground level), an intermediate number of colonies was observed. Consistent with previous trials, most colonies consisted of spore aggregations. *F. poae* consistently produced higher colony numbers than *F. culmorum*.

Discussion

Splash dispersal of spores plays a significant role in the dissemination of fungal pathogens in the field. Furthermore, *Fusarium* spp. are a significant cause

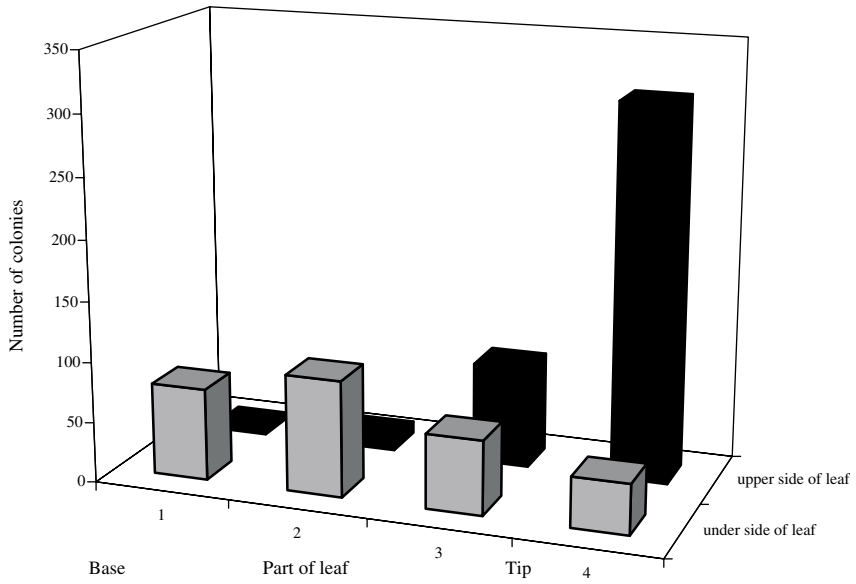

Figure 3. Mean number of *F. poae* colonies on artificial leaves positioned 10 cm above the ground and 20 cm from the original inoculum source, following splash dispersal of spores by waterdrops released from a height of 6 m.

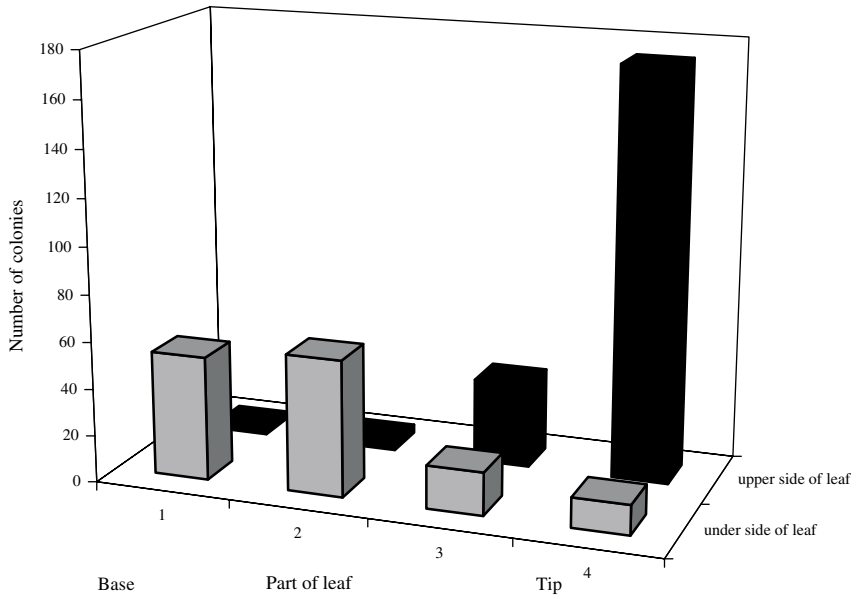

Figure 4. Mean number of *F. culmorum* colonies on artificial leaves positioned 10 cm above the ground and 20 cm from the original inoculum source, following splash dispersal of spores by waterdrops released from a height of 6 m.

of yield losses worldwide. By using a previously described method (Jenkinson and Parry, 1994), splash dispersal patterns onto agar strips were observed for *F. culmorum* and *F. poae*. In addition, a novel method for investigating the pattern of splash dispersed conidia onto leaves under controlled conditions is described.

The patterns of *F. culmorum* dispersal from infected straw onto agar strips correlated with earlier results (Jenkinson and Parry, 1994). It appears that spore morphology does not influence the splash dispersal patterns. Although the conidia of *F. poae* and *F. culmorum* differ in shape, each displayed

similar patterns of spread. This is reminiscent of earlier studies, comparing the morphologically dissimilar *Pseudocercosporella herpotrichoides* and *Pyrenopeziza brassicae* (Fatemi and Fitt, 1983). Furthermore, consistent with our study, previous studies also showed a steep splash dispersal gradient (Brennan et al., 1985; Fitt and Lysandrou, 1984; Fitt et al., 1988; Jenkinson and Parry, 1994; Pedersen et al., 1994).

Using transparency film to form the body of an artificial leaf overlaid with a thin film of SNA successfully allowed patterns of splash dispersed conidia to be assessed. The use of a thin metal thread to stabilise each leaf may have made them too inflexible, since dispersal patterns from inoculated leaves did not differ from infected straw placed on plain surfaces. However, the former presented colonies that generally arose from single spores instead of spore masses. It is likely that the fungus sporulates less on artificial leaves than on straw and thus less spores are spread. It would be expected that spores dispersed onto leaves by water droplets hitting the ground would be mainly dispersed onto the leaf tips that are closer to the source than the base. This predicted dispersal pattern was observed on the upperside of artificial leaves in this trial. Interestingly, however, on the underside of the leaves both pathogens showed the opposite gradient. This phenomenon could be explained by acknowledging that the leaf base, on the underside of the leaf, is at an angle more difficult for droplets to reach, compared to the basal part of the leaf.

Under field conditions, it is thus possible, that spores on the underside of the leaf could easily infect the stem base, while spores on the upperside of the leaf could be further spread by continuous splash events, eventually leading to infection of the head, resulting in *Fusarium* head blight. Nevertheless, the use of artificial leaves as a tool for the analysis of splash dispersed fungal spore patterns was successful. In the future it will be important to study the interactions between growing leaves and conidial spread, and to determine whether dispersal patterns are static or if they follow the dynamics of the growing leaf.

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