Plant Pathology (1999) **48**, 472–482

Dry-dispersal and rain-splash of brown (*Puccinia recondita* f.sp. *tritici*) and yellow (*P. striiformis*) rust spores from infected wheat leaves exposed to simulated raindrops

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The dispersal of spores from lesions of brown (*Puccinia recondita* f.sp. *tritici*) or yellow (*P. striiformis*) rusts of wheat by impacting drops was studied. Using a generator of uniform-size drops, drops of 2·5, 3·4, 4·2 and 4·9 mm in diameter were released from rest at heights of 5, 50 and 100 cm above horizontal and primary leaves uniformly covered with sporulating lesions. Dry-dispersal and rain-splash occurred simultaneously in response to drop impaction. A coloration technique allowed separate counting of dry-dispersed and rain-splashed spores caught on slides. More spores were rain-splashed than dry-dispersed. Neither removal mechanism affected *in-vitro* germination of spores, which was higher in brown than in yellow rust. For both rusts, the number of both dry-dispersed and rain-splashed spores, as well as their travel distance, increased with drop diameter and fall height. The fall speed of incident drops in relation to diameter and fall height was obtained by solving numerically the equation of vertical drop motion. The number of spores removed by a given impacting drop was found to be a power function of the calculated kinetic energy of the impacting drop. Based on this experimental relationship, a simulation study showed the relevance of rain type in the removal of spores.

Keywords: epidemiology, kinetic energy, *Triticum aestivum*, spore dispersal gradient

Introduction

In many rust fungi, mature asexual spores responsible for the spread of epidemic disease form a floccose powder of detached spores (Hirst, 1961) and are removed by passive mechanisms. Cereal rust spores were earlier thought to be dry-removed only by wind (Chester, 1946), but water drops of various origins, e.g. rain and overhead irrigation (Rapilly, 1991), can also cause passive spore removal. The relative importance of wind compared with rain for removal has yet to be assessed in most cases. Various experimental evidence shows, however, that the importance of rain has been underestimated. Spore removal was enhanced by raindrops in *Prunus* (*Tranzschelia discolor*) and snapdragon (*Puccinia antirrhini*) rusts (Carter *et al*., 1970a,b). Rain has also been found to play a major role in the dispersal of coffee leaf rust (*Hemileia vastatrix*) spores, in which the highest spore catches and seasonal development were related to regional rainfall pattern (Kushalappa & Eskes, 1989). On wheat, spores produced by black (*P. graminis* f.sp. *tritici*), brown (*P. recondita* f.sp. *tritici*) or yellow (*P. striiformis*) rust were dry-dispersed by the impact of simulated raindrops (Hirst, 1961; Rapilly, 1979). In field studies, yellow rust occurrence was closely associated with the amount of rain recorded (Park, 1990), and brown rust spores were found in rain collected early in the growing season (Rowell & Romig, 1966).

Both dry-dispersal and rain-splash of spores may be enhanced by rain. Dry-dispersal occurs when a raindrop strikes a dry surface causing an energy transfer, which disturbs the air within the laminar boundary layer and dislodges spores (van der Wal, 1978). Rain-splash occurs when a raindrop strikes a wet or dry surface followed by a transfer of energy to both leaf and splash droplets (Huber *et al*., 1998). Dry-dispersed spores remain dry whereas rain-splashed spores are carried by the water droplets formed. Both mechanisms have been experimentally reproduced with various rust fungi (Hirst & Stedman, 1963; Pauvert *et al*., 1970; Rapilly *et al*., 1970). In these studies, simulated drops of uniform size falling from a given height were used. The studies were limited to one fungus at a time, and dry-dispersal and rain-splash were studied in independent experiments. Moreover, no attempt was made to relate number of dispersed spores to the physical characteristics (kinetic energy or momentum) of the incident drops.

The aim of the experiments reported here was to quantify the dispersal of spores of brown and yellow

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Accepted 9 March 1999.

rusts of wheat in relation to the impact of single simulated raindrops. Dry-dispersal and rain-splash were evaluated in controlled conditions with wheat seedlings at the primary leaf stage as the plant model. Dispersal was characterized by the number of spores removed and their travel distance from the source. Viability of dispersed spores was assessed using germination tests. Several combinations of drop diameter and fall height were tested. Different drop diameters matching the natural raindrop size distribution were used. The vertical distance travelled by the raindrops released from different heights created drops with a range of kinetic energies. The main outcome of this paper is to establish a relation between the quantity of dry-dispersed and rain-splashed spores and the kinetic energy of raindrops. Results are used to discuss the possibility of assessing spore dispersal by rain-splash in natural rainfall conditions.

Materials and methods

Host, fungi and inoculation procedure

Plants bearing sporulating lesions were produced as described elsewhere (Geagea *et al.*, 1997). Briefly, one-leaf seedlings of the susceptible wheat cv. Michigan Amber were inoculated in a settling tower with (uredo)spores of either brown (*Puccinia recondita* f.sp. *tritici*) or yellow (*P. striiformis*) rust using a density of 270 ± 30 spores cm^{-2}. After incubation for 24 h in controlled conditions conducive to infection, seedlings were transferred to a growth room with a 16:8 h light:dark cycle. The temperature was 17 ± 1°C during the light period (light intensity of 250 $\mu E\, m^{-2}\, s^{-1}$ at 1 m from the source) and 15 ± 1°C during the dark period. The experiments were conducted with 13-day-old lesions for *P. recondita* f.sp. *tritici* and 17-day-old lesions for *P. striiformis*. The inoculation procedure created a uniform inoculum density and all seedlings inoculated with a given pathogen were assumed to have produced the same quantity of spores at a given time after inoculation.

Spore dispersal by single incident drops

Drop generator

A 50-mL syringe (Plastipak®10: Becton-Dickinson, Madrid, Spain) was filled with tap water and connected through a polyethylene tube 0·5 cm in diameter to a vertically-held hypodermic needle (Microlance®3 or Terumo®: Becton-Dickinson). A syringe-pump (A99 model: Bioblock Scientific, Illkirch, France) generated single water drops of constant diameter through the hypodermic needle. The volume of drops generated by a given needle was evaluated by weighing 100 drops collected in a small vial and their diameter was estimated from the volume, assuming them to be spherical.

Drops of 2·5, 3·4, 4·2, and 4·9 mm in diameter were generated. These drops simulate the large drops occurring in natural rainfall (Ulbrich, 1983). Drops larger than 5 mm in diameter generally break up before reaching target and drops smaller than 2·5 mm in diameter cannot be targeted accurately (Fitt *et al.*, 1989). The water flow in the syringe-pump allowed drops with the largest diameter to fall every 2 s. The frequency of fall of smaller drops was kept constant by clamping the polyethylene tube to reduce the water flow. Drops of a given diameter fell vertically over heights of 5, 50 and 100 cm. Incident drops released from low heights (< 40 cm) simulate canopy drip (Yang *et al.*, 1991).

Target leaf

A primary leaf bearing sporulating lesions, the 'target leaf', was held horizontally 5 cm above a sampling tray. The petiole was held in a PVC spiral and the leaf tip was fixed to a microscopic slide with plasticine. Therefore, the target leaf was allowed to flutter when impacted by water drops (Fig. 1). This set-up did not exactly mimic the natural position of the leaf but was highly reproducible.

For a given combination of drop diameter and fall height, drops were released in groups of three, up to a total of 18 drops; preliminary experiments showed that no additional spore removal occurred with further drops. The target leaf was changed after each experiment with a given drop diameter and fall height combination. Each combination was replicated three times.

Spore collection and analysis of spore dispersal

Spores released through the impaction of three water drops were collected on glass slides (76 × 26 mm) coated with a thin layer of naphthol green B solution (4 g L^{-1}), prepared by adding dye powder (Siegfried SA, Zofingue, Switzerland) to boiling water (modified from Liddell & Wootten, 1957). The dye allowed dry-dispersed and rain-splashed spores to be distinguished (Rapilly *et al.*, 1970). Each splash droplet collected, carrying spores or not, left a circular spot with a discoloured centre and a defined dark green margin on the slide surface. In

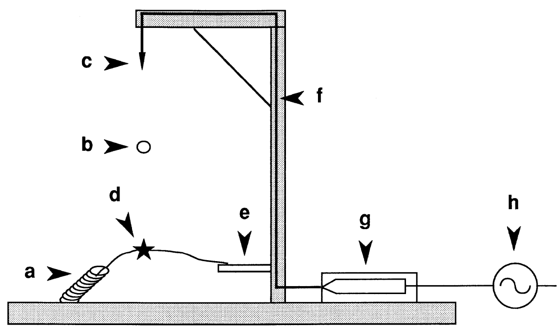


Figure 1 Schematic representation of the single drop generator. (a) PVC spiral holding the leaf petiole; (b) incident drop; (c) hypodermic needle; (d) target leaf bearing sporulating lesions (impaction point marked with a star); (e) glass slide for attaching the leaf tip; (f) polyethylene tube connecting the needle to the syringe-pump; (g) syringe-pump; (h) power supply.

contrast, dry-dispersed spores caused no discoloration of the slide surface. Slides were laid horizontally 5 cm below the target leaf. One slide was laid just below the expected impaction point and its centre was further considered as 'the centre'. Other slides covered the whole horizontal surface within 12 cm of the centre in order to collect all the removed spores. Preliminary experiments showed that no spores were collected beyond 9 cm. Counting was done using an optical microscope (magnification ×100). As the spore distribution on slides was not spatially homogenous, all collected dry-dispersed and rain-splashed spores were counted. Each collecting slide was characterized by the distance of its own centre from the centre of the experiment as defined above. All spores collected on a given slide were summed and assumed to have travelled the distance between the impaction point and the centre of the slide.

For each type of dispersal mechanism, each fungus and each set of three drops of a given diameter and released from a given height, the log-transformed number of deposited spores was related to the distance d (cm) by a logarithmic relationship:

$$\ln(y) = a - bd \qquad (1)$$

where:

y is the total number of removed spores found at distance d (cm);

a and b are the intercept and slope parameters of the linear regression, respectively.

Spore germination

The germination rate of dry-dispersed and rain-splashed spores was assessed as follows. The syringe of the drop generator was filled with rainwater instead of tap water to simulate natural conditions. Rainwater was collected in plastic boxes (60×40×30 cm) 1 week before the experiment and sieved through a filter paper (Whatman #40: OSI, Elancourt, France). Three drops of each of 2·5, 3·4, 4·2 and 4·9 mm diameter were released from a height of 50 cm onto the target leaf. The released spores were collected on glass slides coated with a solution (5 g L^{-1}) of agar-agar (OSI) including a few drops of naphthol green B. Exposed slides were put in Petri dishes covered with wetted filter paper and enclosed in polyethylene bags. Germination was induced for 24 h in darkness at 15°C for *P. recondita* f.sp. *tritici* (Clifford & Harris, 1981) and 8°C for *P. striiformis* (Rapilly, 1979). The experiment was repeated twice. All germinated and ungerminated dry-dispersed and rain-splashed spores found on a slide were counted; numbers ranged between 340 and 1500. Spores were considered germinated if the germ tube was longer than the spore diameter (approximately 30 μm for both rusts).

Characteristics of single drops and rainfall

Kinetic energy of single incident drops

In order to relate spore removal to a physical characteristic of the simulated drops, the kinetic energy (J) of drops was calculated ($KE = 1/2\,mv^2$, where m is mass of the drops in kg and v their vertical velocity in m s^{-1}). Runge–Kutta–Merton integration (NAG FORTRAN library routine D02BBF: Numerical Algorithms Group, 1990) was used to calculate the vertical velocity of a free-falling drop, diameter D (m) fall height h (m), in still air (Huber *et al.*, 1997). The kinetic energy was calculated from:

$$KE(D) = 0{\cdot}5\rho\pi D^3 v^2/6 \qquad (2)$$

Where ρ is the density of water (10^3 kg m^{-3}).

Integral parameters of rainfall

Based on the results obtained under the experimental conditions described above, the effect of drop diameter and kinetic energy on the total number of removed spores was investigated for both rusts with several hypotheses on natural raindrop size distribution. With the assumption of a terminal velocity v (m s^{-1}) given by $386{\cdot}6\,D^{0{\cdot}67}$, where D is in metres (Ulbrich, 1983), the kinetic energy of one drop is given by:

$$KE(D) = 0{\cdot}5\rho\pi(D^3/6)(386{\cdot}6D^{0{\cdot}67})^2$$
$$= 39{\cdot}1\times10^6 D^{4{\cdot}34} \qquad (3)$$

The number of drops of a given diameter $N(D)$ (m^{-3} cm^{-1}) per unit volume of air and per unit size interval having equivolume spherical diameter D (m) is given by Ulbrich (1983):

$$N(D) = N_0 D^{\mu} \exp(-\Lambda D) \qquad (4)$$

with Λ (cm^{-1}) $= \varepsilon$ $(3{\cdot}67 + \mu)$ $R^{-\delta}$ (R = rainfall rate, mm h^{-1}). Parameters N_0 (m^{-3} cm^{-1}), μ (dimensionless), ε (dimensionless) and δ (dimensionless) are specific to the type of rain. For three types of rain (thunderstorm, shower and widespread) with $R = 10$ mm h^{-1}, the values of the parameters were chosen according to Ulbrich (1983). For the thunderstorm type, N_0, μ, ε and δ were $2{\cdot}05\times10^6$, 1·63, 0·13 and 0·16, respectively. For the shower type, N_0, μ, ε and δ were $9{\cdot}2\times10^{10}$, 5·04, 0·129 and 0·1, respectively. For the widespread type, N_0, μ, ε and δ were $6{\cdot}4\times10^{10}$, 4·65, 0·114 and 0·11, respectively. The number of incident drops (number of impactions) and their momentum or the rainfall power (kinetic energy per time interval) can be calculated by integration of the complete or truncated drop size distribution (Huber *et al.*, 1998). Based on number of incident drops and kinetic energy per 5 min, an analysis of the effect of rainfall type on spore removal by rain-splash is developed in the discussion section.

Results

Effect of increasing number of single incident drops

The experiments showed that both brown and yellow rust spores can be removed by water drops despite their reputation of being nonwettable (Rapilly, 1991). The impact of a raindrop falling on a leaf bearing sporulating

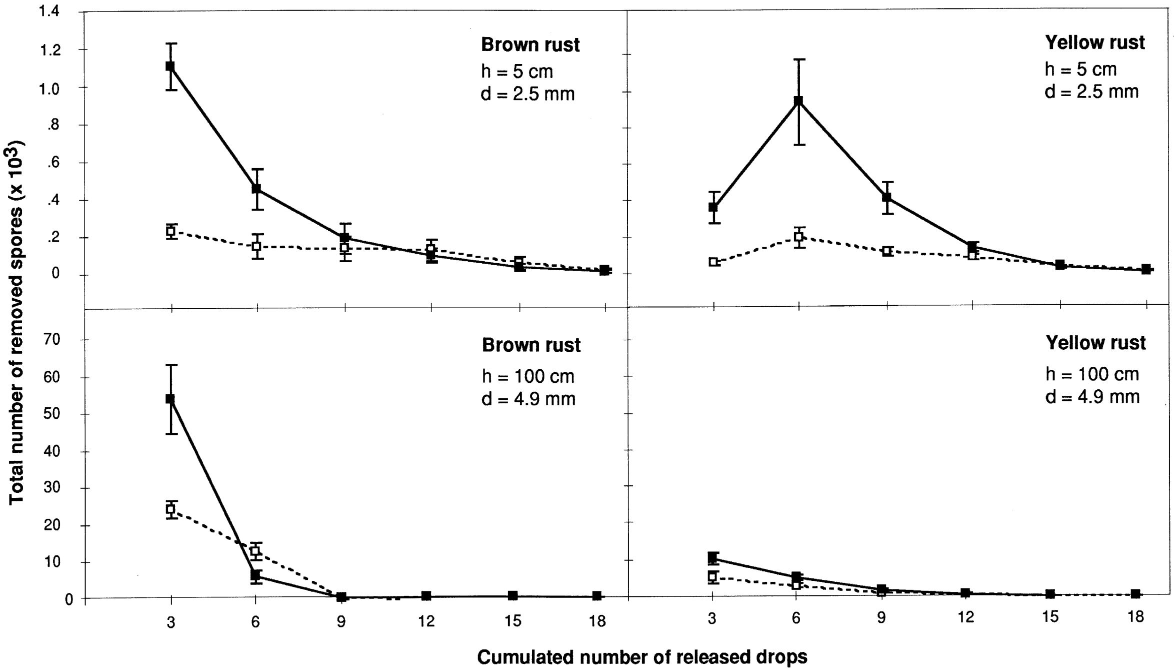


Figure 2 Effects of increasing number of incident water drops on the removal of spores in brown and yellow rusts of wheat. Top panels: drops of 2·5 mm in diameter were released from a height of 5 cm. Bottom panels: drops of 4·9 mm in diameter were released from a height of 100 cm. Collecting slides were changed after every three successive drops. Dry-dispersed (open squares, dotted lines) and rain-splashed (filled squares, solid lines) spores were counted separately (see text for details). Means and standard errors of three repetitions are given.

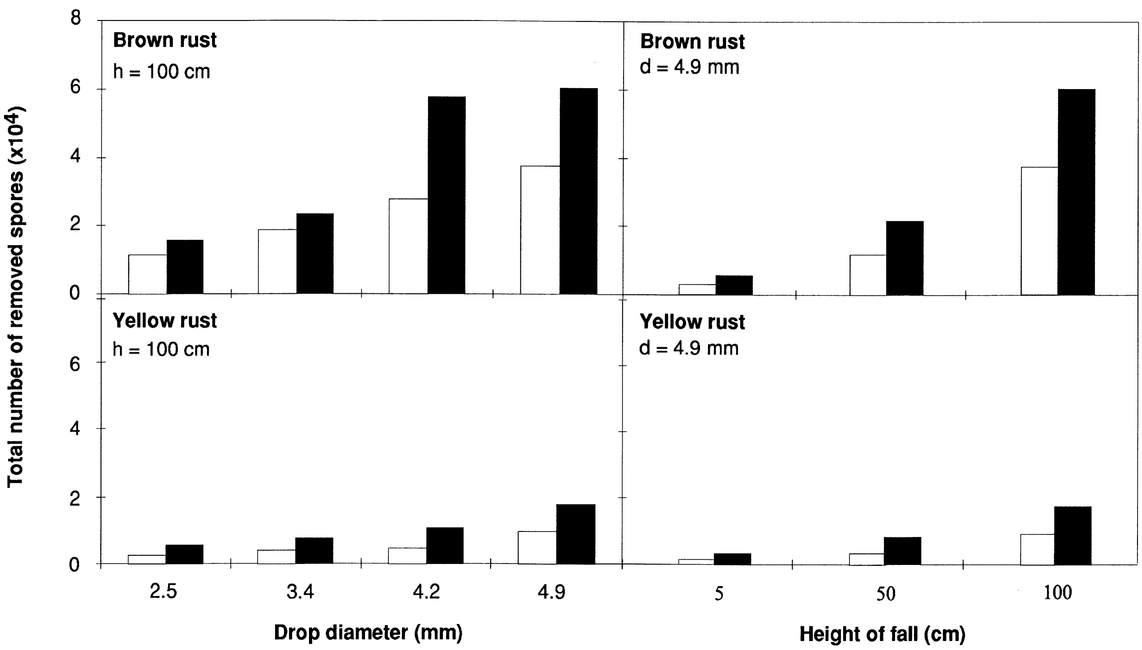


Figure 3 Effects of increasing water drop diameter (left panels) and fall height (right panels) on the removal of spores in brown and yellow rusts of wheat. Dry-dispersed (open bars) and rain-splashed (filled bars) spores were counted separately (see text for details). Each bar represents the cumulated number of removed spores collected over all distances after 18 drops.

lesions resulted in the collection of many dry-dispersed and rain-splashed spores on slides placed at different distances and in all four directions. After the first three drops, the number of rain-splashed spores collected was greater than the number of dry-dispersed ones for both rusts and for all combinations of drop diameter and fall height. The number of spores collected per three drops, either dry-dispersed or rain-splashed, decreased with the increase in the number of impacting drops. Exhaustion of lesions occurred after the release of 18 drops for all drop diameter and fall height combinations. In the case of brown rust and for all combinations, 50–80% of dry-dispersed and rain-splashed spores were removed after the first three drops. This result was also obtained for yellow rust except for drops of 2·5, 3·4 and 4·2 mm in diameter released from 5 cm; in these three combinations, the numbers of dry-dispersed and rain-splashed spores peaked after the second three drops and then decreased to become very low ($\approx$ 0·1–3%) after 18 drops (Fig. 2). Total number of dry-dispersed and rain-splashed spores was much greater in brown than in yellow rust, for example $5{\cdot}5 \times 10^4$ for brown rust and $1{\cdot}2 \times 10^4$ for yellow rust for a drop of 4·9 mm in diameter falling from 100 cm. This trend occurred for all combinations (drop diameter $\times$ fall height). Therefore, results obtained with drops of 4·9 mm in diameter released from 100 cm were chosen to be representative of the whole experiment (Fig. 2).

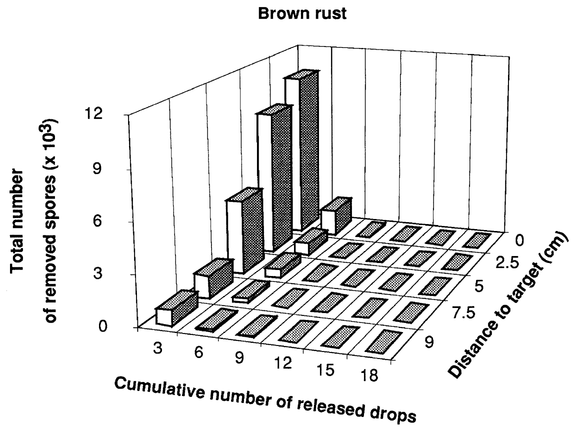
Effects of drop diameter and fall height

The total number of removed spores of *P. recondita* f.sp. *tritici* and *P. striiformis* and their travel distance increased with increasing drop diameter and fall height. For a given fall height, total number of dry-dispersed and rain-splashed spores increased with increasing drop diameter: for a height of 100 cm, the number of spores removed under the impact of a drop of 4·9 mm in diameter was approximately three times greater than the number removed under the impact of a drop of 2·5 mm in diameter, for both brown and yellow rusts (Fig. 3). For a given drop diameter, the number of dry-dispersed and rain-splashed spores of both brown and yellow rusts increased with increasing fall height: for a drop diameter of 4·9 mm, 6×10^4 and $0{\cdot}8 \times 10^4$ spores were removed for fall heights of 100 and 5 cm, respectively, in brown rust (Fig. 3). The travel distance of spores also increased with increasing drop diameter and fall height. The maximum travel distance observed throughout the experiment was 9 cm from the source. No spores were found beyond 2·5 cm from the source when drops of 4·9 mm in diameter were released from 5 cm; drops of the same diameter but released from 100 cm caused spores to travel up to 9 cm. When a drop of a smaller diameter (2·5 mm, for example) was allowed to fall from this same height, no spores were found beyond 7·5 cm from the source. Results obtained with the combination 4·9 mm drop diameter and 100 cm fall height were thought to be illustrative for the whole experiment (Fig. 4).

The number of removed spores collected at different distances decreased with distance from the source. Fitting a linear model after log-transformation (Eqn 1, Tables 1 and 2) showed that for the various drop diameter and fall height combinations, dispersal gradients from the inoculum source were very similar, i.e. the slopes (b) of linear regression did not differ significantly between the different combinations ($P = 0{\cdot}05$). The number of spores found on the slide laid below the expected point of impaction increased with increasing drop diameter and fall height and decreased with number of released drops.

Germination of removed spores

Spores of both fungi were able to germinate on water agar independently of drop diameter and fall height.



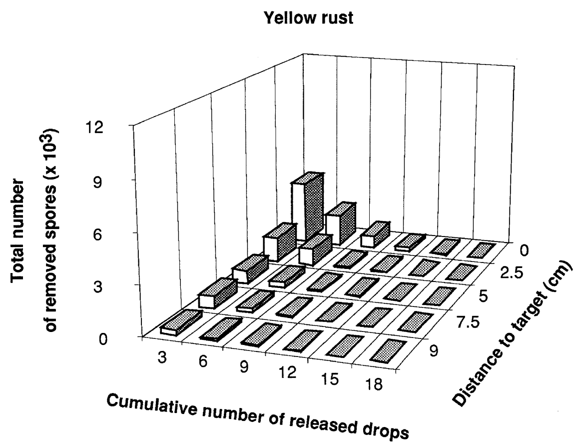


Figure 4 The spore dispersal gradient in brown and yellow rusts of wheat after the impact of water drops of 4·9 mm in diameter and released successively from a height of 100 cm. Collecting slides were changed after every three successive drops. Both dry-dispersed and rain-splashed spores were cumulated over all directions. No spores were collected beyond 9 cm.

Table 1 Results of linear regression of log-transformed number of deposited spores of *Puccinia recondita* f.sp. *tritici* against distance from impaction point after impaction of *n* drops of diameter *D* falling from height *h*

Drop characteristics			Dry-dispersed			Rain-splashed		
D (mm)	*n*	*h* (cm)	*a*	*b*	R^2	*a*	*b*	R^2
2·5	3	5	4.54	0.12	0.74	6.68	0.82	0.97
		50	6.65	0.19	0.73	7.04	0.22	0.84
		100	7.59	0.19	0.86	7.81	0.18	0.80
	6	5	4.10	0.15	0.72	5.76	0.66	0.83
		50	4.98	0.14	0.71	6.24	0.33	0.73
		100	5.65	0.11	0.75	6.95	0.25	0.84
	9	5	4.05	0.18	0.72	4.32	0.31	0.88
		50	4.27	0.13	0.78	5.48	0.33	0.73
		100	4.72	0.05	0.71	5.29	0.27	0.80
	12	5	3.88	0.17	0.79	4.17	0.64	0.71
		50	3.55	0.11	0.77	3.87	0.29	0.70
		100	3.66	0.13	0.77	4.00	0.29	0.81
	15	5	3.11	0.20	0.79	3.07	0.47	0.56
		50	3.02	0.13	0.80	2.58	0.18	0.68
		100	2.49	0.15	0.73	2.78	0.13	0.63
3·4	3	5	5.95	0.53	0.97	6.80	0.51	0.66
		50	6.65	0.20	0.69	7.43	0.28	0.64
		100	8.18	0.29	0.77	8.86	0.39	0.88
	6	5	4.96	0.33	0.87	6.24	0.52	0.75
		50	5.83	0.25	0.65	6.86	0.39	0.71
		100	7.33	0.25	0.75	7.64	0.33	0.81
	9	5	4.44	0.34	0.89	5.60	0.64	0.77
		50	4.83	0.28	0.62	5.55	0.36	0.79
		100	5.56	0.17	0.80	6.16	0.30	0.85
	12	5	3.49	0.18	0.66	5.13	0.76	0.84
		50	3.33	0.13	0.67	4.06	0.38	0.68
		100	4.98	0.25	0.79	5.26	0.30	0.71
	15	5	2.44	0.18	0.59	4.23	0.70	0.76
		50	2.80	0.07	0.62			
		100	4.13	0.39	0.86	2.84	0.28	0.71
4·2	3	5	5.99	0.14	0.63	7.41	0.95	0.92
		50	7.55	0.37	0.83	8.28	0.41	0.82
		100	8.16	0.15	0.81	9.08	0.21	0.80
	6	5	5.39	0.09	0.77	6.38	0.71	0.93
		50	6.21	0.26	0.83	6.54	0.28	0.73
		100	6.71	0.12	0.77	7.68	0.17	0.78
	9	5	5.16	0.15	0.68	5.39	0.67	0.82
		50	4.64	0.18	0.70	5.63	0.27	0.76
		100	5.37	0.12	0.79	6.09	0.10	0.77
	12	5	4.44	0.15	0.65	4.75	0.76	0.69
		50	3.72	0.21	0.73	4.15	0.25	0.71
		100	3.77	0.09	0.66	3.67	0.17	0.71
	15	5	3.49	0.19	0.66	4.12	0.63	0.95
		50	2.75	0.15	0.63	2.65	0.18	0.76
		100	2.83	0.23	0.75			
4·9	3	5	6.83	0.22	0.80	7.55	0.54	0.79
		50	7.31	0.14	0.76	8.32	0.22	0.83
		100	8.20	0.14	0.87	9.77	0.32	0.87
	6	5	5.54	0.04	0.79	6.82	0.57	0.78
		50	6.49	0.17	0.79	6.67	0.11	0.76
		100	7.39	0.09	0.66	7.59	0.32	0.79
	9	5	4.09	0.01	0.70	6.46	0.96	0.77
		50	4.79	0.09	0.66	6.05	0.50	0.80
		100	4.42	0.24	0.63	3.97	0.21	0.61
	12	5	3.26	0.07	0.68	4.45	0.69	0.81
		50	3.82	0.24	0.85	4.76	0.42	0.79
	15	5	2.13	0.17	0.65	3.39	0.67	0.66

a, Intercept; *b*, slope; R^2, coefficient of determination.

Table 2 Results of linear regression of log-transformed number of deposited spores of *Puccinia striiformis* against distance from impaction point after impaction of *n* drops of diameter *D* falling from height *h*

Drop characteristics			Dry-dispersed			Rain-splashed		
D (mm)	*n*	*h* (cm)	*a*	*b*	R^2	*a*	*b*	R^2
2·5	3	5	2.90	0.09	0.74	5.28	0.38	0.74
		50	5.65	0.21	0.73	7.26	0.54	0.83
		100	5.95	0.21	0.71	7.23	0.39	0.77
	6	5	4.83	0.29	0.75	6.39	0.55	0.73
		50	4.20	0.28	0.80	6.46	0.48	0.76
		100	5.36	0.19	0.74	6.39	0.41	0.80
	9	5	3.90	0.15	0.42	5.61	0.63	0.78
		50	2.93	0.34	0.77	5.44	0.42	0.83
		100	4.42	0.23	0.80	5.51	0.37	0.87
	12	5	3.75	0.27	0.72	4.56	0.62	0.85
		50	2.28	0.30	0.73	4.43	0.34	0.72
		100	3.27	0.33	0.82	4.48	0.36	0.69
	15	5	3.22	0.77	0.93	3.17	0.44	0.90
		50				3.34	0.25	0.61
		100	2.24	0.46	0.96	3.38	0.26	0.75
3·4	3	5	4.67	0.61	0.80	5.64	0.39	0.80
		50	6.14	0.43	0.78	7.68	0.65	0.86
		100	6.47	0.26	0.83	7.69	0.39	0.88
	6	5	5.83	0.89	0.96	6.87	0.68	0.92
		50	4.20	0.15	0.61	6.73	0.58	0.89
		100	5.70	0.23	0.72	6.99	0.38	0.79
	9	5	4.41	0.77	0.97	5.73	0.53	0.85
		50	3.79	0.14	0.73	5.92	0.61	0.91
		100	5.08	0.21	0.76	5.76	0.37	0.78
	12	5	3.44	0.51	0.89	4.32	0.25	0.78
		50	3.36	0.22	0.68	4.90	0.54	0.76
		100	4.68	0.35	0.76	4.44	0.26	0.88
	15	5	2.87	0.44	0.57	3.56	0.60	0.75
		50	2.69	0.25	0.76	3.59	0.51	0.71
		100	3.15	0.13	0.59	3.31	0.43	0.82
4·2	3	5	5.38	0.59	0.80	5.31	0.22	0.79
		50	6.46	0.40	0.87	7.69	0.46	0.91
		100	7.21	0.46	0.92	7.42	0.29	0.82
	6	5	5.77	0.38	0.81	7.10	0.83	0.86
		50	5.11	0.26	0.89	6.52	0.44	0.87
		100	5.94	0.39	0.89	7.13	0.34	0.86
	9	5	4.57	0.12	0.56	6.22	0.76	0.93
		50	4.15	0.27	0.89	4.95	0.33	0.87
		100	4.97	0.32	0.89	6.21	0.38	0.89
	12	5	3.39	0.28	0.61	4.63	0.44	0.82
		50	3.57	0.31	0.89	4.25	0.37	0.89
		100	3.58	0.20	0.78	5.07	0.31	0.91
	15	5	2.89	0.23	0.61	3.19	0.35	0.62
		50	3.04	0.39	0.79	3.71	0.39	0.75
		100	2.98	0.21	0.82	3.92	0.34	0.81
4·9	3	5	6.19	0.46	0.72	7.43	0.86	0.97
		50	6.21	0.19	0.83	7.98	0.43	0.93
		100	7.58	0.34	0.84	8.03	0.24	0.84
	6	5	5.68	0.38	0.69	6.26	0.69	0.94
		50	5.51	0.17	0.84	6.95	0.41	0.94
		100	6.96	0.34	0.79	7.61	0.31	0.92
	9	5	3.56	0.23	0.61	5.06	0.45	0.72
		50	4.83	0.15	0.73	5.44	0.31	0.82
		100	5.71	0.26	0.88	6.02	0.24	0.78
	12	5	3.32	0.60	0.67	3.86	0.32	0.78
		50	4.39	0.21	0.67	4.09	0.20	0.81
		100	4.66	0.23	0.84	5.18	0.28	0.91
	15	5	2.58	0.23	0.63	2.83	0.31	0.69
		50	3.47	0.16	0.67	3.02	0.21	0.72
		100	3.27	0.17	0.83	3.51	0.29	0.92

a, Intercept; *b*, slope; R^2, coefficient of determination.

Table 3 Fall speed (v) and kinetic energy (KE) of water drops falling from rest in relation to drop diameter (D) and fall height (h). Values were calculated as explained in text

h (m)	D ($\times 10^{-2}$ m)	v (m s^{-1})	KE ($\times 10^{-6}$ J)
0·05	2.5	0.96	4
	3.4	0.96	9
	4.2	0.96	18
	4.9	0.97	29
0·5	2.5	2.96	36
	3.4	3.03	94
	4.2	3.07	183
	4.9	3.09	294
1	2.5	4.04	67
	3.4	4.14	176
	4.2	4.22	345
	4.9	4.26	559

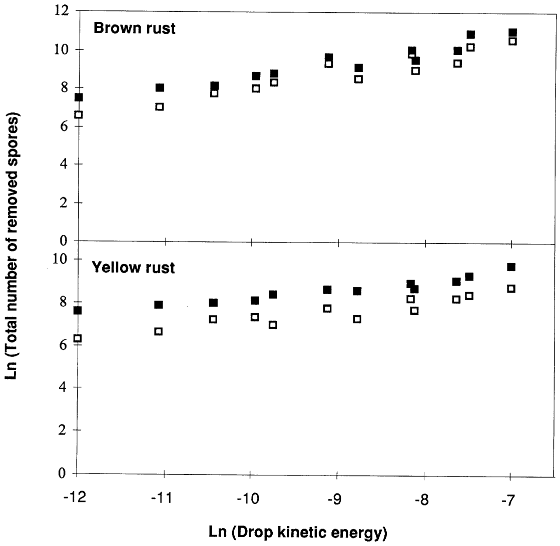


Figure 5 The relationship, after log-transformation, between calculated kinetic energy of impacting water drops and removal of dry-dispersed (open squares) and rain-splashed (filled squares) spores in brown and yellow rusts of wheat.

Germination rate did not differ between dry-dispersed ($\approx$ 70–97%) and rain-splashed ($\approx$ 73–96%) spores ($P > 0{\cdot}05$). Germination rate was higher for brown rust ($\approx$ 95%) than for yellow rust ($\approx$ 70%: $P = 0{\cdot}05$).

Effect of the kinetic energy of single incident drops on spore dispersal

The combined effect of drop diameter and fall height on the number of spores dispersed was characterized by kinetic energy of drops at impact (Eqn 2). The fall speed and kinetic energy of drops at impact are listed in Table 3. The number of spores increased with kinetic energy for both pathogens and types of dispersal (Fig. 5). A power-law relationship between the number of spores removed (S) and drop kinetic energy (KE) was fitted to these data:

$$\text{Brown rust, dry-dispersed}: S = e^{16{\cdot}04} 0{\cdot}76 \quad (5a)$$

$$\text{Brown rust, rain-splashed}: S = e^{15{\cdot}95} \times KE^{0{\cdot}69} \quad (5b)$$

$$\text{Yellow rust, dry-dispersed}: S = e^{12{\cdot}03} \times KE^{0{\cdot}46} \quad (5c)$$

$$\text{Yellow rust, rain-splashed}: S = e^{12{\cdot}42} * KE^{0{\cdot}40} \quad (5d)$$

Slopes of the linear regressions were not significantly different between the two dispersal mechanisms for each rust ($P > 0{\cdot}05$). Slopes of the linear regressions differed significantly between the two rusts for each dispersal mechanism ($P = 0{\cdot}05$).

Integral parameters of rainfall

Using Eqn 3, the terminal velocity (v) of natural raindrops was calculated as well as the kinetic energy (KE) of each raindrop. For a 5-min period of rain, the number of impacting drops and kinetic energy for the drops of the three rain types were deduced. Based on these calculations and the power law relationship between number of rain-splashed spores and rain kinetic energy (Fig. 5), the total number of potentially rain-splashed spores of both fungi (Fig. 6b) was calculated assuming a constant rain intensity of 10 mm h^{-1} for each rain type (Fig. 6a). The simulations assume that inoculum is evenly spread on horizontal leaves; this is not the case in reality and therefore, although the simulations are used to compare the qualitative effects of different rain types, the numbers cannot be taken as actual estimates of spore removal rates. The power law relationship is experimentally valid for kinetic energy less than 559 μJ (corresponding to a drop of 4·9 mm in diameter falling from a height of 1 m). For drops falling at terminal velocity, this corresponds to a drop diameter less than 3·2 mm. Simulations were made with the following assumptions: no splash by drops less than 1 mm in diameter (zone I, hatched, in Fig. 6b), a power law relationship assumed to be valid for drop diameters between 1 and 3·2 mm (zone II in Fig. 6b), and an extrapolated power law relationship for a drop diameter greater than 3·2 mm (zone III in Fig. 6b). With a chosen rain intensity of 10 mm h^{-1}, the concentration of drops in the size range 1–1·5 mm increases from the thunderstorm to the widespread type of rain (Fig. 6a), which explains the same hierarchy on Fig. 6(b) for these diameters. In the size range 1·5–2·0 mm, the hierarchy is thunderstorm, widespread and shower, which leads to the same rank on Fig. 6(b) with considerable differences between rusts due to differences in removal efficiency. In the size range 2·0–3·2 mm, the thunderstorm type has most incident drops. The very high removal efficiency of these large drops leads to vast numbers of spores being removed. Total calculated numbers of spores potentially

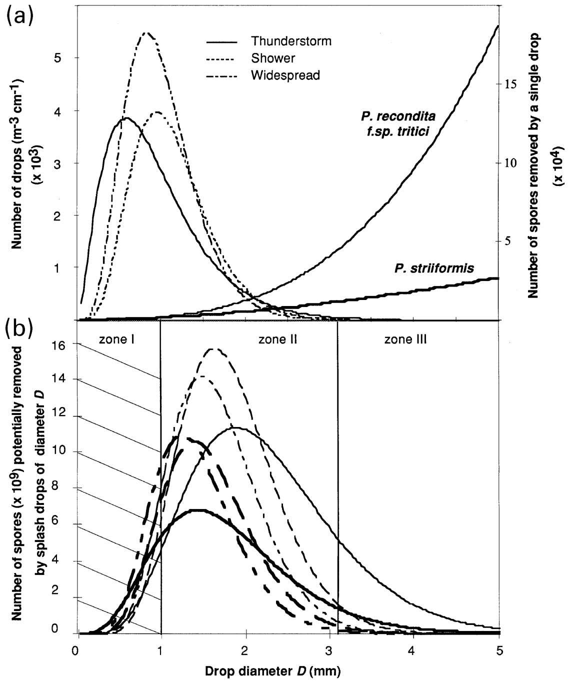


Figure 6 Simulation of splash-dispersal of spores of brown and yellow rusts by incident drops of given diameters produced by three types of rain. (a) Drop size distribution for three rain types (thunderstorm, shower and widespread) according to Ulbrich (1983) and the number of spores removed by one single drop of a given diameter. (b) Number of brown (bold lines) and yellow (normal lines) rust spores removed by incident drops produced by the three types of rain (as described in Fig. 6a). The drop diameter interval is 0·0025 mm, rain duration is 5 min and rainfall intensity is 10 mm h^{-1}. The three zones depicted relate to the interval in which the power law relationship between number of removed spores and kinetic energy is valid: zone I (hatched), no relationship – no splash; zone II, experimental validity interval of Eqn 5; zone III, extrapolation of Eqn 5 beyond the experimental validity interval.

removed for both rusts during 5 min of rain with intensity of 10 mm h^{-1} ranged from 10^{10} to $2{\cdot}5^{10}$.

Discussion

Simulated raindrops removed spores of *Puccinia recondita* f.sp. *tritici* and *P. striiformis*, although cereal rusts are known to be essentially wind-dispersed (Rapilly, 1979, 1991). Rain may be involved in the removal of other typically wind-dispersed spores either by dry-dispersal or rain-splash. In the latter case, nonwettable spores will be picked up directly in rain-splash droplets striking the spore-bearing surfaces (Ramalingam & Rati, 1979) and carried away on the surface of the splash droplets (Fitt & McCartney, 1986). The present results confirm the rain-dispersal of spores reported for other rusts (Hirst, 1961; Carter *et al.*, 1970a,b; Kushalappa & Eskes, 1989). In the present study, the two mechanisms of dry-dispersal and rain-splash occurred simultaneously and nonexclusively. Slides coated with naphthol green B simultaneously trapped dry-dispersed and rain-splashed spores of both fungi, as already reported by Rapilly *et al.* (1970) for yellow rust only. The number of spores removed decreased with cumulated number of impacting drops (Fig. 2) for both fungi, indicating lesion exhaustion as already reported from more limited experiments (Hirst & Stedman, 1963; Rapilly *et al.*, 1970). At the start of experiments, drops remove spores of *P. recondita* f.sp. *tritici* and *P. striiformis* accumulated at the surface of the sorus. When approaching exhaustion, raindrops remove spores located deeper within the sorus.

Drop diameter and fall height strongly affected spore removal (Fig. 3). Large drops (4·9 mm) removed four times as many spores as smaller ones (2·5 mm), and both kinds of drop removed more spores when they fell from 100 cm rather than from 5 cm. A similar trend was reported for spores of *Lycopodium* (Hirst & Stedman, 1963), *Fusarium solani* (Gregory *et al.*, 1959) and *Rhynchosporium secalis* (Fitt *et al.*, 1989), and sporangia of *Phytophthora cactorum* (Reynolds *et al.*, 1989; Yang *et al.*, 1992). This trend is explained by the increase in drop kinetic energy associated with increasing drop diameter and fall height, causing an increase in the number of dry-dispersed and rain-splashed spores (Fitt & McCartney, 1986). Comparison of the relationships obtained for the two rusts and the two dispersal mechanisms (Fig. 5, Eqn 5a–d) suggests that lesion morphology, which differs between the two rusts, affects rain-induced dispersal. The number of dry-dispersed and rain-splashed spores deposited on a horizontal surface decreased rapidly with increasing distance from the inoculum source, and few spores travelled beyond 9 cm (Fig. 4). Rain-induced dispersal in still air causes a steeper deposition gradient than eddy diffusion of dry airborne spores (Stedman, 1980). Deposition gradients of rain-splashed spores are usually steeper than those of wind-dispersed spores where few rain-splashed spores travel beyond 1 m (Gregory, 1973; Fitt & McCartney, 1986; Madden, 1992).

Differences in the total number of spores removed were noticed between the two fungi and in some cases the peak in removal of *P. striiformis* spores was found after six drops (Figs 2 and 3). For a given pathogen, the inoculation procedure led to standard spore production for all experiments. Spore production per leaf is quite similar for the two rust fungi (Sache & de Vallavieille-Pope, 1993) and cannot explain large differences in number of spores removed. Spores of *P. striiformis* are embedded in a thin mucilaginous layer, whereas spores of *P. recondita* f.sp. *tritici* remain isolated (Rapilly & Fournet, 1968). A greater kinetic energy was required to remove the larger dissemination units (spore clusters) (Rapilly, 1977) of *P. striiformis* than those of *P. recondita* f.sp. *tritici* (single spores). A similar conclusion was drawn from spore removal experiments done with either a miniaturized wind-tunnel or a centrifuge (Geagea *et al.*, 1997). When it starts raining, the first drops wet the surface, render soluble the mucilage holding the

P. striiformis spores, and allow them to float over a water film, from which they may be either dry-dispersed by direct impact of raindrops or rain-splashed into the air when a raindrop strikes the water film (Gregory *et al*., 1959).

While the largest raindrops falling at terminal velocity appear most effective in rain-dispersal (Fitt & McCartney, 1986), the present experiments with fall heights between 5 and 100 cm showed that dry-dispersal and rain-splash can also occur with large drops falling at a velocity lower than their terminal velocity. Such drops can occur during both overhead irrigation and as drip drops produced by rain modification by the canopy (Gregory *et al*., 1959; Stedman, 1979; Yang *et al*., 1991). Dripping drops may have sufficient kinetic energy to produce splash droplets and drip-splash can be an important means of spore dispersal (Fitt & McCartney, 1986).

Rain was characterized by drop kinetic energy rather than drop momentum, which could also have been related to spore dispersal, because of the future availability of an electric sensor for measuring the kinetic energy of impacting raindrops in natural conditions (Madden *et al*., 1998). The range of kinetic energy values obtained in the experimental conditions used here (Eqn 2) corresponds to natural drops of small diameter (2–3·2 mm: Eqn 3). These small drops with reduced kinetic energy are usually more numerous than larger ones in real rainfall and, while they do not contribute to disease spread on a large scale, they may contribute to the dispersal of spores from one leaf to another lower and relatively protected leaf. Reynolds *et al.* (1987) showed that, for typically wind-dispersed spores, the impact of raindrops or drip drops may be a prerequisite for removal of inoculum held in a water film.

The simulation of dispersal by rain-splash emphasizes the relevance of variation in drop diameter when considering real rainfall (Fig. 6b). Drops with diameters less than 1 mm are omitted, because they bounce and do not splash (Huber *et al*., 1997). As expected from Eqn 5b and d, more spores are potentially removed for brown rust than for yellow rust. For increasing drop diameter (greater than 1 mm), qualitative differences between rain types result from the concurrent decrease in drop size frequency distribution and increase in removal efficiency (Fig. 6a). When integrating the whole spectrum of drop sizes, total simulated numbers of spores potentially removed indicated that variations in drop size distribution and spore removal efficiency in relation to drop diameter do not show large overall differences between rain types.

The experiments reported here were undertaken with a simple biological model, i.e. a primary leaf bearing sporulating lesions and held horizontally. In more realistic conditions, other characteristics would obviously affect dry-dispersal and rain-splash of spores. The spore-bearing structure affects the rate of spore dispersal (Pauvert *et al*., 1970). Leaf orientation, crop age and changes in leaf texture and flexibility induced by the pathogen affect the two dispersal mechanisms in the case of *Pseudocercosporella capsellae* (Fitt *et al*., 1992). Surface microtopography characteristics such as cuticle roughness and leaf geometry also have a major effect on rain-induced dispersal (Madden, 1997; Huber *et al*., 1998).

A very important issue is whether these findings still remain applicable in changing conditions of fungal inoculum source or rain. The effect of inoculum source, for example depletion, and the effects of rain duration and intensity on the efficiency of splash-dispersal require further investigation. In field conditions, the question of the relative importance of both dry-dispersal and rain-splash should be addressed with reference to differences in dispersal behaviour between brown and yellow rusts.

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