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The spread of apple brown rot (*Monilinia fructigena*) by insects

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Summary

Field and laboratory experiments were carried out to compare the spread of apple brown rot (*Monilinia fructigena*) by free aerial transmission of conidia, with insect spread of the pathogen. Significantly more brown rot developed on injured apples which had been visited by insects than on unvisited ones. The results obtained suggest that insects play an important role in aetiology of the disease, especially under conditions unsuitable for abiotic spread.

Introduction

The importance of insects in the spread of certain plant diseases is well known and the subject has been extensively reviewed (Rand & Pierce, 1920; Leach, 1940; Metcalfe, Flint & Metcalfe, 1962; Carter, 1973; Agrios, 1980). The interactions between insect and plant pathogen range from specific symbiosis and vectoring, through to more casual relationships in which insect dissemination may be only one of a number of means of spread. Here, the importance of insect activity may often be overlooked or underestimated.

The brown rot fungi (*Monilinia* spp.) are pathogens of top fruit, dispersed principally by means of conidia produced in pustules on the surface of infected fruit and overwintered fruit mummies (Byrde, 1952). They rely almost exclusively on pre-existing wounds in the fruit skin for penetration (Moore, 1951; Wormald, 1954), *Monilinia fructigena* (Aderh & Ruhl). Honey is a common pathogen of apples and plums in Europe. A variety of dispersal mechanisms may operate, including rain splash, dispersal in air currents and transport by insects. It has been suggested or assumed that wind dispersal and rain splash may be the main mechanisms of dispersal, but the relative importance of insect vectors is not known (Byrde & Willetts, 1977; Lack, 1985).

Materials and Methods

Three sets of experiments were conducted in an orchard in Somerset, England, to investigate the relative importance of insect vectoring of brown rot compared with wind dispersal and a series of laboratory wind tunnel experiments were carried out to study *M. fructigena* spread (at low wind speeds) in the presence and absence of insects.

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Field Experiments

Exposure of artificially wounded apples

Eleven experiments were carried out during September 1984, each comprising a set of six Golden Delicious apples which each had a slit 2 cm long cut into the skin immediately before being brought into the orchard. (Golden Delicious were used throughout as a standard fruit available all year round, to allow comparison with the results of other experiments). Six orchard apples, fully infected with brown rot, had previously been collected and placed in two rows of three and the Golden Delicious were placed between these, slits uppermost, to give two rows of six apples, alternately infected and uninfected. Each row of apples was 50 cm long. These apples were then observed closely from a point 0.5 m away, for between 10 min and 1 h, depending on the amount of insect activity in the orchard, (which varied considerably, according to weather conditions: on days when large numbers of landings were made on the apples, experiments were terminated before the full hour, in order to have some 'unvisited' left). All visits made by insects to the apples and to the wounds in the Golden Delicious were noted. The six infected apples were there to provide a high local inoculum level and in addition all experiments were conducted under trees known to be infected with brown rot, to prevent the results from being biased away from free aerial spread of the fungus. Very few insects which landed on the Golden Delicious did so immediately after a visit to one of the six infected apples, so the vectoring component of the spread was not being artificially enhanced. At the end of the observation period, the Golden Delicious were collected and placed in individual marked pots, stored at room temperature for 1-3 wk (depending on temperature) and subsequent development of brown rot recorded.

Exposure of agar plates

In August and September 1984 a total of 10 sets of 20.5 cm diameter prune-juice-agar (PJA) plates were exposed in the orchard under an infected tree for 1 h. In every case, the plates were set out close together, over an area of about 0.25 m^2, and any insect visits made to each plate were noted. The two experiments on 18 September were not simultaneous. At the end of the hour, the plates were collected up and stored at room temperature for 1 wk. Any fungal colonies at all similar in growth form or colour to *Monilinia fructigena* were marked and plated onto two replicates of fresh PJA plates and at the end of a further week all the resulting colonies which resembled *M. fructigena* were tested on two pieces of a Golden Delicious apple; the third piece of each apple was kept as a control. From the results of these inoculations, the number of brown rot colonies developing on each of the original PJA plates was obtained, and hence the rate of colony production m^{-2} h^{-1} was found.

Exposure of half apples

On three occasions in September 1984, 20 halves of Golden Delicious apples were exposed adjacent to the PJA plates described above. These were observed concurrently with the agar plates and all insect visits recorded, and were then placed in individual marked pots and stored at room temperature for 1-3 wk. Development of brown rot on the apples' halves was then recorded.

Laboratory Experiments

A series of nine experiments was carried out, being three replicates of each of three wind speeds. Each experiment used two wind tunnels, one containing 50 laboratory reared adult *Drosophila subobscura* and the other with no flies. The wind tunnels were 100 cm long and had a square cross-section of 30 cm side. The tunnels were fitted with narrow wires at 7.5 cm intervals down their length, against which the apples used for the infection studies were

placed. These wires served both to prevent the apples from rolling into each other and also to space them out evenly down the tunnels. Each tunnel contained 10 apples (Golden Delicious) 9 of which were uninfected but with a fresh wound 1 cm long cut into the upper surface; the tenth apple was fully infected with brown rot and placed in the middle of the tunnel (position five from the down-wind end) to act as a source of inoculum.

The wind tunnels were supplied with extractor fans of various sizes, linked to one end of the tunnels and drawing air through the muslin panels on their end doors. The extractor fans were arranged to expel air through a tube leading out of the experimental room, to prevent contamination of the air stream by brown rot conidia. The three wind speeds used were 'still air' 0.1 km h^{-1} 0.2 km h^{-1} measured in the centre of the tunnel. Relative humidity was monitored using hair hygrometers placed inside the tunnels.

For each experiment, a set of apples was placed in each tunnel, the batch of flies was added to one of the tunnels, and the extractor fans were put on for 5 days; the number of infected apples per tunnel was then noted. All experiments were conducted under a 12:12 h light:dark regime, at 20°C ± 2.5°C.

Results

Field Experiments

Brown rot development on apple tissues

Significantly more brown rot development occurred on apple tissues visited by insects than those tissues which were not visited (Table 1) (wounded apples:- $\chi^2 = 20.6$ with 1.d.f. $P < 0.001$.Apple halves:- $P = 0.0026$; Fisher's exact test).

Brown rot colony growth on agar plates

These experiments were primarily assessing free aerial spread of brown rot, since the plates are not intended to be attractive to insects (compare lines 3 and 4 of Table 1). No brown rot was detected by these experiments until late in the season (Table 2) when the disease was already well established in the orchard. Five out of the nine colonies which developed were on plates which had not been visited by insects, the remaining four colonies may either have been insect vectored or aerially spread. Assuming that all nine colonies were aerially spread, a mean development rate of colonies m^{-2} h^{-1} can be calculated from:

$$n/a\pi r^2 t$$

where n = number of brown rot colonies which developed
a = number of plates used
r = radius of the plates = 0.025 m
t = number of hours of exposure

For the last four experiments, when brown rot was being detected by this method, this gives an upper estimate of a mean development rate of 57.3 colonies m^{-2} h^{-1}. For the final experiment, with the highest colony formation data, the corresponding figure is 178 colonies m^{-2} h^{-1}. If we assume an average wound size in fallen apples of 1 cm^2, that none of these colonies were insect vectored and an even settling of viable conidia both in time and over the ground, these figures correspond to a mean infection time by free aerial dispersal of 175 hours per apple, under prevailing orchard conditions.

The insect groups involved

Identification to species was not possible from the observation points, but insect visitors

Table 1. *Development of brown rot on different substrates visited and unvisited by insects*

Substrate type	Total no. substrates exposed	Exposure time (minutes)	Number visited	Number unvisited	Number (and percentage) of 'visited' developing rot	Number (and percentage) of 'unvisited' developing rot	Total number of visits
wounded apples	66	29 (mean)*	47	19	33 (70.2%)	5 (26.3%)	200
prune juice agar (pja) (total)	200	60	50	150	3 (6%)	2 (1.33%)	65
pja when half apples also used	60	60	7	53	1 (14.3%)	2 (3.8%)	8
half apples	60	60	30	30	10 (33.3%)	1 (3.3%)	62

* exposure times in experiments using wounded apples ranged from 10 minutes to an hour, depending on the level of insect activity in the orchard.

Table 2. *Development of brown rot colonies on prune juice agar plates visited and unvisited by insects*

Date of experiment	7/8	9/8	20/8	21/8	3/9	6/9	am 10/9	pm 10/9	am 18/9	am 18/9
No of plates which developed brown rot:										
'visited'	0	0	0	0	0	0	1	1	0	1
'unvisited'	0	0	0	0	0	0	0	0	0	2
total	0	0	0	0	0	0	1	1	0	3
Total no. of brown rot colonies:										
on 'visited' plates	0	0	0	0	0	0	1	1	0	2
on 'unvisited' plates	0	0	0	0	0	0	0	0	0	5
total	0	0	0	0	0	0	1	1	0	7
No. of plates visited out of a possible total of 20:	3	1	12	11	8	4	4	4	2	1

in all three sets of experiments were identified to Order and where possible to Family. The main groups visiting both apple tissues and agar plates, and therefore most likely to be vectors of the rot, were Diptera (especially Muscidae, Syrphida and *Drosophila* spp., and Hymenoptera (Parasitica, Vespidae and Apoidea).

Laboratory Experiments

Twenty five out of a possible total of 81 apples became infected in the tunnels with flies, but only one became infected with the absence of flies, and this one was 'upwind' of the source of inoculum. This is a very highly significant result in favour of transmission being carried out by the flies ($P = 0.0002$; Fisher's exact test). No effect of increasing wind speed was found. The full results for each wind speed are shown in Table 3.

Table 3. *Results of wind tunnel transmission experiments in the presence and absence of flies*

Wind speed in the tunnel	replicate	Relative humidity (%) in the tunnel	*No. of apples which became infected*	
			+ 50 flies	no flies
0	1		3	0
	2	60-70	3	0
	3		1	0
0.1 kmh-1	1		2	0
	2	57-74	2	0
	3		6	1
0.2 kmh-1	1		4	0
	2	50-60	2	0
	3		2	0

Discussion

The results presented here suggest that insects especially Diptera and Hymenoptera, have an important role in the spread of *M. fructigena*. The wind tunnel experiments suggest that *M. fructigena* is not well adapted to rapid aerial transmission. Even in September in the orchard, when the rot was well advanced in fallen fruit and damaged fruit in the trees, free aerial spread did not appear to be a major component in the dispersal system.

It has often been assumed in the past (Byrde, 1952; Kable, 1965; Jenkins, 1965; Corbin, Ogawa & Schultz, 1968; Byrde & Willetts, 1977) that aerial dispersal is a major means of spread of brown rots of several species, although it has been recognised that the viability of the thin-walled, hyaline conidia may be adversely affected by both low humidity and ultra-violet light. Kable (1965), suggests that this may be a major factor limiting aerial dispersal of *Monilinia fructicola* in Australia. The other important limiting factor is the availability of suitable substrates, since the fungus is almost wholly reliant on wounds in the fruit skin for penetration sites. These two aspects of aerial dispersal – the survival and availability of inoculum and the landing of spores on suitable substrates – probably largely account for the low levels of aerial spread actually observed here, both in the field and in the wind tunnels.

In spite of the limitations of the wind tunnels as models of the field system, the results presented here suggest that at very low wind speeds, *M. fructigena* is largely spread by insects or other means, e.g. rain splash, and not air currents. The conidia of *M. fructigena* are large (*c.* 20 μm × 12 μm) and although they are held above the skin of infected fruit there is no active release of spores and the conidiophores are short; these factors will all tend to limit further the free aerial dispersal of the fungus.

It is to be expected that insects will be relatively more effective means of dispersal of the fungus at low wind speeds: not only are the insects then capable of free taken-off and direct flight, but numbers of brown rot conidia in the air spora are reduced in low winds (Jenkins, 1965; Kable, 1965; Corbin, *et al.*, 1968). Ambient windspeeds in the orchard used for field work in the current studies were usually 3 km h^{-1} and although not measured, canopy and phylloplane windspeeds will have been correspondingly lower (see for example, Corbin, *et al.*, 1968).

Taken on their own, the results of the wind tunnel experiments are of limited used in assessing field spread of *M. fructigena* by insects; the conditions of wind speed, relative humidity and temperature used in these experiments are all approximately those found in an orchard in late summer, but the laboratory system did not allow for the effects of dew, rain, nor for microclimatic variations in humidity, nor for the succession of fresh and therefore moist wounds which are made in fallen apples in the field and which are the principal entry sites for the fungus. But together with the field results, they point to the relative unsuitability of *M. fructigena* for dispersal by abiotic means, and suggest that insect spread may be more important than previously thought.

Insects have occasionally been cited as possible vectors of the brown rots (Wormald, 1954; Willison & Dustan, 1956; Ogawa, 1957; Kable, 1969; Tate & Ogawa, 1975), but their relative importance has not previously been assessed. Insects have certain clear advantages over wind currents as dispersal mechanisms: they can act selectively both in picking up inoculum and in depositing it at suitable sites, they can reduce the passage time from source to new substrate, and they can in some cases afford physical protection to the spores being carried. *Drosophila subobscura* both feeds and breeds in rotting and healthy fruit (Lack, 1985), but any insect visiting both rotting and healthy fruit will be liable to act as a casual carrier of the fungus. The evidence presented here suggests that this happens with such frequency that insects are a very important factor in the spread of the disease.

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