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Tuber blight in relation to *Phytophthora infestans* genotype

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SUMMARY
National incidences of tuber blight were generally low during the years that genotype 13_A2 dominated the GB population. This study examined if lower than expected tuber blight was related to the aggressiveness of this genotype. Isolates of 13_A2 were generally more aggressive on tubers than isolates of older genotypes (but there was considerable variation within genotypes). This result implies that greater problems with tuber blight should have been experienced in the years that 13_A2 dominated. There is some evidence to support the idea that 13_A2 causes a higher incidence of tuber blight initially during the growing season but due to faster decay of tubers infected with 13_A2 the incidence of tuber blight at harvest could be lower than for less aggressive genotypes. The experiments demonstrated that not only did the more aggressive 13_A2 colonise tuber tissue more quickly than isolates of older genotypes but the severity of secondary bacterial soft rotting was significantly greater for 13_A2. The more rapid soft rotting associated with 13_A2 requires more detailed experimental work.

KEYWORDS
Late blight, *Phytophthora infestans*, tuber blight, 13_A2

INTRODUCTION

The national incidence of tuber blight was low in recent years (2007 to 2011). This coincided with the domination of the GB *P. infestans* population by isolates of the 13_A2 genotype. It isn't clear whether this was simply a coincidence or whether there was a causal relationship between genotype of *P. infestans* and the incidence of tuber blight, with isolates of genotype 13_A2 resulting in low incidences. This study examined the influence of *P. infestans* isolate on tuber blight using two different direct inoculation methods. The first attempted to simulate the natural infection process in the field. The second was a more artificial point inoculation method. In addition the impact of isolate on the period of survival of blighted tubers was investigated.

MATERIALS AND METHODS

Tuber infection in situ in relation to P. infestans genotype (2011)

Six chitted seed tubers (20-30 mm diam.)(cv. Rocket) were shallow planted at a depth of 5 cm on 13 April 2011 in 49-litre tubs containing John Innes No 1 compost. Four replicate tubs were planted. Each tub was labelled with replicate and isolate. The tubs were placed in a polytunnel for 15 weeks to encourage rapid tuber initiation and development and to keep the foliage dry.

Tap water (130 litres) was stored at room temperature for at least 48 hours. This was used for watering the tubs after they were moved to the controlled temperature room. Another c. 130 litres were stored at 10°C for watering the inoculum into the compost on the day of inoculation. The haulm from each tub was cut c.10 cm above the compost surface to allow the tubs to be stacked in the controlled temperature room. The compost was saturated with the tap water, which had been stored at room temperature, to ensure that tuber lenticels were open prior to inoculation and the tubs incubated on 25 July 2011 for 24 hours at 4°C. Tubs were watered from below and overhead so that the compost was wet throughout the complete depth profile.

P. infestans isolates were grown on King Edward potato leaves for 10 days at 16°C, 16-hour day length. Sporangial suspensions were prepared by washing leaves with a 9:1 mixture of sterile distilled water and potato tuber extract (McKee, 1964) that had been pre-incubated at 16°C for 24 hours. This temperature was to prevent zoospores being produced prior to the time of tuber inoculation. Once the concentration of sporangia for each test isolate was determined, the isolate with the lowest concentration was eliminated and replaced with a *P. infestans*-free control (water only) to check for natural infection. The concentration of sporangia for individual isolates was adjusted to 8.25 x 10^{4} sporangia in 100 ml per tub. To assess direct and indirect germination, aliquots of the sporangial suspensions were incubated at 10°C for 24 hours, then fixed and assessed.

All leaf material was removed from the surface of the compost and solid trays were kept in place below the tubs during the inoculation process to prevent cross contamination between tubs. The inoculum suspensions (100 ml) were dribbled evenly onto the surface of the compost using Sarstedt plastic disposable cups with a small hole drilled in the bottom of each cup. A clean cup was used for each isolate. Immediately after inoculum was dribbled onto the surface, sporangia were watered in using 5.4 litres of tap water per tub. The water had been stored at 10 ^{o}C for 24 hours. Tubs were stacked two high and arranged as a randomised complete block. Two days after inoculation, trays were drained of any water to alleviate anaerobic conditions, particularly at the base of the tubs. The temperature of the room was increased to 10°C and the tubs were incubated for 21 days. The progeny tubers were harvested, washed, dried and destructively assessed to determine the incidence of blight. Four replicates were assessed, however only the results from two were analysed because of severe bacterial soft rot in tubs of the other two reps. The bacterial soft rot was sufficiently severe to prevent accurate assessment of tuber blight. A similar experiment was carried out in 2012.

Determine the influence of P. infestans genotype on the survival of inoculated tubers (2011)

Tubers (cv. Saxon) were washed, dried and surfaced sterilised. Tubers were placed in rows on trays containing damp tissue. Each tuber was wounded once using a cork borer of 15 mm diameter (sterilised between rows with IMS and flaming), which was pushed into the tuber to a depth of 5 mm. A random sample of intact tubers was tested for contamination by

Pectobacterium atrosepticum and/or *Pectobacterium carotovorum* subsp. *carotovorum*. A total of eight isolates of *P. infestans* and five replicates were used. Four replicates were for tuber burial in the field whilst the fifth replicate was used to determine blight severity in relation to isolate (destructive assessment). In total 100 tubers per isolate were inoculated (20 tubers per replicate).

P. infestans isolates were grown on King Edward potato leaves for 7 days at 16°C, 16-hour day length. Sporangia were washed from the leaflets using sterile distilled water, the concentration was adjusted and 20 µl of sporangial suspension (containing 20 sporangia) were point inoculated into each wound site on 15 September 2011. Individual suspensions were thoroughly mixed between each row of tubers using a Gallenkamp® Spinmix (The Technology Centre, Loughborough, UK). Control tubers were inoculated with sterile distilled water only. Trays containing the inoculated tubers were labelled, placed in large black bags and the bags sealed before being stored at 4°C and high relative humidity for 15 days (Table 1). The black bags were removed after 7 days. Trays were arranged as a randomised complete block. Following the incubation period, trays were placed in an ambient store for 7 days prior to planting.

Due to heavy and persistent rainfall towards the end of September 2011, burial was delayed to allow ground conditions at the site to improve. To limit disease progress prior to planting, tuber samples were stored at 4°C for a further 4 days. Tubers from replicate five were transferred to the ambient store, 24 hours prior to destructive assessment, to encourage disease development. Replicate five was destructively assessed on 12 October 2011. Tubers were cut transversely through the wound/inoculation point and each tuber assessed for blight severity (%).

***Table 1**.* *Details of dates and timings (2011 experiment)*

Task	Date
Inoculation	15 September
Incubation (4°C)	15 - 30 September
Bags removed	22 September
Incubation (12°C)	30 September – 07 October
Incubation (4°C)	07 – 11 October
Incubation (12°C) (Rep. five)	11 – 12 October
Burial (South Holm)	11 October

Before burial each tuber was individually weighed and numbered. Tubers were transferred to the field in their individual trays and laid out next to the relevant plot. Each plot was 3.4 m (4 rows) x 7.50 m with 2 m spacing between plot ends and 2.6 m between plot sides. Tubers were hand planted in the middle two rows to a depth of 14.5 cm with 20 cm spacing between tuber centres, using a randomised complete block design (four replicate blocks). Trowels were thoroughly washed and gloves changed between plots to prevent cross contamination. Fifteen extra tubers were inoculated with isolate 2008_6082F (13_A2) to allow monitoring of tuber rotting throughout the period of burial. Two weeks after burial five tubers were individually harvested and destructively assessed to monitor disease progression. All tubers were harvested by hand 6 weeks after burial (21 and 22 November 2011) and individual tubers were washed thoroughly to remove all soft rotted tissue and each tuber re-weighed. Tubers were also assessed for the

presence or absence of blight symptoms. A similar experiment was carried out in April to June, 2012.

RESULTS AND DISCUSSION

Tuber infection in situ in relation to P. infestans genotype (cv. Rocket 2011)

In the 2011 experiment, on average 13_A2 isolates caused a significantly higher incidence of tuber blight than representative isolates of the old population (Table 2). However, there was considerable variation between isolates of 13_A2 (Fig. 1). Only one 6_A1 isolate was included in the experiment due to the poor growth of 6_A1 isolates prior to inoculation. The 6_A1 isolate tested gave the lowest incidence of infection. This isolate was grouped with the 13_A2 ones to compare new genotypes with old. The new genotypes resulted in a higher incidence of tuber infection but the difference was just statistically significant (Table 2). Similar results were obtained in the 2012 experiment (data not shown).

Table 2. *Mean in situ infection for isolates of old and new genotypes, cv. Rocket, 2011*

	13_A2	6_A1	Old
	2008_6082F	2008_6090A	2008_7006D (2_A1)
	07/39		2009_7122A (7_A1)
	2009_7654A		2008_6422F (8_A1)
		New (13_A2 and 6_A1)	
Mean infection (%)	29.2	23.8	14.7
F pr. 13_A2 vs. Old	0.008		
F pr. New vs. Old	0.046		

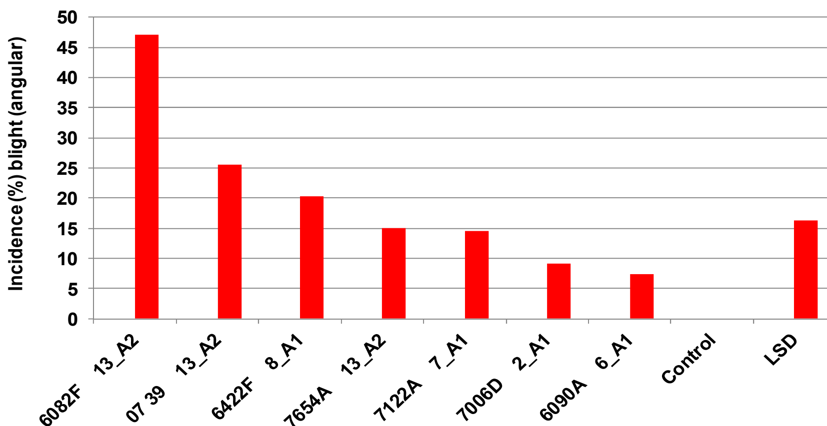

Figure 1. *Incidence of in situ infection of cv. Rocket tubers by different isolates of P. infestans, 2011*

Compared with the old isolates the 13_A2 isolates tested resulted in a significantly higher *in situ* infection (14.7% and 29.2% respectively) and yet the 13_A2 isolates had significantly lower indirect germination percentages (28.6% and 23.4% respectively) and direct germination percentages (15.5% and 10.4% respectively).

Determine the influence of P. infestans genotype on the survival of inoculated tubers

In both experiments, 2011 and 2012, tubers infected with 13_A2 isolates developed significantly more severe tuber blight than those inoculated with older genotypes (Tables 3 and 4). The presence of genotype 13_A2 also accelerated bacterial soft rotting (Tables 3 and 4). This result was anticipated because it is known that blight infection of tubers predisposes them to secondary rot by bacteria (Sicilia *et al.*, 2002). However, the absence of a strong relationship between tuber blight severity and soft rot severity was not expected. The weak relationship was partly due to limited soft rot development for some isolates in relation to the tuber blight severity they caused, e.g. isolate 2008_7006D in the 2011 experiment (Fig. 2). Less severe bacterial soft rot than expected was generally limited to isolates of the older genotypes, i.e. 2008_7006D in the 2011 experiment and 2008_7006D, 2010_8042B and 2008_6422F in the follow-up experiment (Fig. 3).

However, although results were consistent in both experiments for isolate 2008_7006D they were not for 2008_6422F. The lack of a consistent result was for soft rot, not blight. This suggests that a factor other than *P. infestans* genotype influenced tuber decay by bacteria. It's assumed that the bacterial soft rot was caused by the *Pectobacterium carotovorum* subsp. *carotovorum* detected in low numbers on non-wounded, non-inoculated tubers. The mean number of bacteria per tuber was 7.0 in the 2011 experiment and 295 in 2012. It's possible that tuber contamination by *Pectobacterium* varied within the tuber stocks used. However, this explanation appears unlikely because the three replicates of each stock tested for contamination gave a similar result. Also, a large number of tubers were inoculated with each *P. infestans* isolate. The *P. infestans* inoculum for tuber inoculation was prepared from inoculated leaf material. This was a deliberate decision to avoid the issue of axenic culture influencing the aggressiveness of isolates on host tissue. However, one drawback of this method is that the concentration of pectolytic bacteria in the *P. infestans* suspensions may have been affected by the condition of the leaves used to prepare the inoculum. A higher concentration of bacteria may have been washed from leaf lesions with more advanced blight development. Further experiments, in which bacterial contamination of sporangial suspensions will be controlled, will re-test the influence of *P. infestans* isolate on the rate of tuber soft rotting.

In the absence of oospores, survival of *P. infestans* between growing seasons is in infected tubers. The above result suggests that the survival of blighted buried progeny tubers is less likely if infected by 13_A2 compared with older, less aggressive genotypes. However, in the one study that specifically examined overwinter survival of inoculated tubers in relation to isolate aggressiveness, survival was unaffected by isolate even although aggressiveness differences were substantial (Montarry *et al.*, 2007).

The more rapid, and more severe, bacterial rotting of tubers infected with 13_A2 compared with older genotypes has a potential implication for seed tuber-borne blackleg. A key early step in blackleg development is rotting of the seed tuber by *Pectobacterium atrosepticum*. If this happens more quickly after planting then either there is a higher incidence of non-emergence (blanking) or, if the plant emerges, blackleg symptoms are likely to develop earlier in the

growing season. Seed crops entered in classification schemes are more likely to be affected because of the low tolerances for blackleg. Tuber blight incidences are normally low therefore any impact on blackleg development in ware crops is likely to be small.

Table 3. *Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of P. infestans and buried in soil, 2011 experiment*

Genotype	13_A2	6_A1	Old
Isolates	2006_3928A	2008_6090A	2008_7006D
	2008_6082F	2009_7126A	2009_7122A
	2009_7654A		2008_6422F
Mean blight severity (%)	26.9	15.7	20.3
Mean soft rot severity (%)	35.5	31.6	28.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	<0.001	0.004	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.002	0.039	

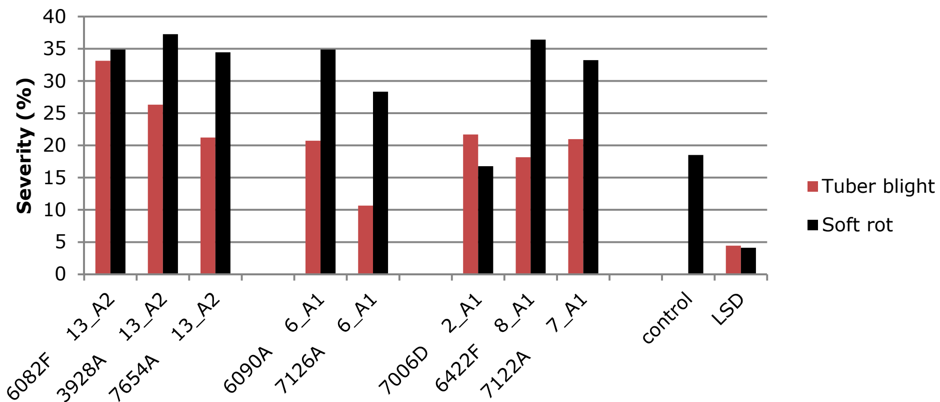

Figure 2. *Severity of blight and bacterial soft rot for tubers inoculated with different isolates of P. infestans and buried in the field, 2011 experiment*

Table 4. *Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of P. infestans and buried in soil, 2012 experiment*

Genotype	13_A2	6_A1	Old
Isolates	2008_6082F	2008_6090A	2008_7006D
	07/39	2011_8406A	2010_8042B
	2008_6102A	2011_8986A	2008_6422F
Mean blight severity (%)	31.4	27.5	24.0
Mean soft rot severity (%)	42.8	46.5	11.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	0.004	0.007	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.009	<0.001	

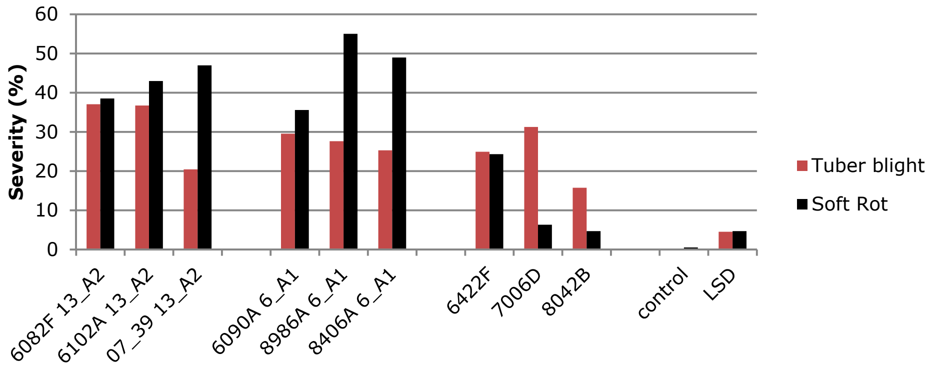

Figure 3. *Severity of blight and bacterial soft rot for tubers inoculated with different isolates of P. infestans and buried in the field, 2011 experiment*

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