WUR – SPECIAL REPORT NO 19 – 2019, 79-86

Integrated control to manage pathogen evolution

FAYE RITCHIE[1], NEIL PAVELEY[2], ISABEL CORKLEY[3], RUAIRIDH BAIN[4], ALISON LEES[5], DAVID COOKE[5], KEVIN CAROLAN[6], FRANK van den BOSCH[7]

[1] ADAS Boxworth, Battlegate Road, Boxworth, CB23 4NN, England, UK
[2] ADAS High Mowthorpe, Duggleby, Malton, YO17 8BP, England, UK
[3] ADAS Wolverhampton, Titan One Offices, Coxwell Avenue, Wolverhampton, WV10 9RT
[4] SRUC, John Niven Building, Auchincruive Estate, Ayr, KA6 5HW, Scotland, UK
[5] James Hutton Institute, Invergowrie, Dundee, Scotland, DD2 5DA, UK
[6] Rothamsted Research, West Common, Harpenden, AL5 2JQ
[7] Department of Environment & Agriculture, Centre for Crop and Disease Management
Curtin University, Bentley 6102, Perth, Australia

SUMMARY
Previously published mathematical modelling has shown that the selection rate for a new virulent or fungicide insensitive pathogen strain is positively associated with the *per capita* growth rate of the population. In the model simulations (which assumed that virulence and fungicide insensitivity were not genetically linked) fungicide treatment slows selection for virulence, by slowing the growth rates of virulent and avirulent strains, thus reducing the difference in their growth rates that drives selection. By the same principles, host resistance that limits epidemic rate should slow the evolution of fungicide insensitivity. Data are presented here from three field experiments to test the evolutionary principles derived by modelling. Selection for a clonal variant (13_A2), insensitive to phenylamides, was measured on two potato cultivars of contrasting partial host resistance, and not treated or treated with different doses of a phenylamide fungicide. Epidemic growth rates varied substantially between experimental sites and seasons, and the rate of selection was significantly slower where the *per capita* growth rate was lower. Virulence and fungicide insensitivity appeared to be linked in 13_A2. The modelling study made the assumption that these factors were not linked, therefore the pathosystem that was tested differed fundamentally from the assumptions in the modelling study. We conclude that the impact of cultivar and fungicide choice to provide acceptable disease control in the field should be considered alongside the impact of the combination of those control methods on the selection for clonal variants. Where a clonal variant already exists (even at very low frequency) which combines virulence and fungicide insensitivity but is not yet detected then integrating the affected host resistance and the affected fungicide mode of action will not help to reduce selection for the clone.

KEYWORDS
Late blight, *Phytophthora infestans*, fungicides, cultivar resistance, integrated control

INTRODUCTION

A key determinant of durability of host resistance is the rate at which one or more virulent strains are selected for in a pathogen population, thus increasing in frequency until control is eroded. Similarly the effective life (durability) of a fungicide mode of action is determined substantially by the rate of selection for insensitive strains. Aggressive and virulent *P. infestans* genotypes, as well as strains with decreased fungicide sensitivity, have been reported for *P. infestans* populations in the last 10 years. The foliar resistance ratings of several cultivars have been downgraded from resistant to moderately resistant (e.g. Cara with resistance rating of 7 in 2010 and 5 in 2012) when exposed to more aggressive and virulent genotypes such as 13_A2 (Lees *et al.*, 2012). Resistance to phenylamides has been widespread since the 1980s (Carter *et al.*, 1982; Holmes and Channon, 1984), and is a trait also associated with genotype 13_A2. More recently, decreased sensitivity to a widely used fungicide, fluazinam, has been reported for a new genotype, 37_A2 (Schepers *et al.*, 2018). A critical question therefore is how to best manage pathogen evolution of virulence and fungicide insensitivity. It is frequently hypothesised that complete reliance on either host resistance or fungicide is likely to be less durable than an integrated approach. The work reported here tested some principles underlying that hypothesis.

Principles governing the selection of fungicide insensitive strains have been derived and tested extensively against experimental data (van den Bosch *et al.*, 2014). These principles show that reducing the difference in the *per capita* rate of increase of sensitive and insensitive strains, slows selection; $s = r_R - r_S$. Resistance management aims to reduce s, the selection coefficient, by slowing the rate of increase of both the fungicide insensitive (r_R) and fungicide sensitive (r_S) strains, for example, by adding a second fungicide mode of action which is effective against both strains. By extension, it can be inferred from the governing principles that: (i) partial (rate-limiting) host resistance, which is effective against fungicide sensitive and insensitive strains should slow selection for fungicide insensitivity, and (ii) fungicide treatment, which is effective against avirulent and virulent strains, should slow selection for virulence. Modelling has demonstrated that this principle could be used to suggest ways to extend the durability of cultivar resistance (Carolan *et al.*, 2017).

For host resistance to reduce the rate of selection for fungicide insensitivity, however, it would need to affect the growth rate of both fungicide sensitive and insensitive strains. The *P. infestans* population in some countries is predominately clonal, resulting in the greater potential for strong genetic linkage between virulence and fungicide resistance in a set of clonal variants. There is therefore a need to understand how integrated control should be deployed. The aims of the work reported in this paper were: (i) to test whether the rate of selection of strains is associated with epidemic growth rate, as predicted by the governing principle, and (ii) to test the effect of integrated control in a pathosystem where virulence and fungicide insensitivity are not genetically linked.

MATERIALS AND METHODS

Field experimental design

In 2015 and 2016, three experiments were conducted to determine the effects of cultivar and fungicide on selection for the *Phytophthora infestans* clonal variant 13-A2. Two experiments were conducted in Ceredigion, Wales and one experiment in Ayrshire, Scotland. Experiments were laid out in a randomised plot design with four replicates. The cultivars were King Edward

(foliar late blight resistance rating 3) and Cara (5) (British Potato Cultivar Database). Six treatments incorporating three levels of fungicide inputs and cultivar resistance to control foliar late blight were included (Table 1). Plots were four rows wide by c. 8m long, or 8 rows by 5.1 m long. The full dose (1.0 dose) of Ridomil Gold 480 SL (480 g/L metalaxyl-M: Syngenta Crop Protection Ltd) to apply was calculated as 0.155 L/ha. This was taken from the maximum individual dose for metalaxyl-M in Fubol Gold (64% w/w mancozeb + 3.88% metalaxyl-M: Syngenta Crop Protection Ltd).

To minimise the risk of infection by inoculum other than the test isolates the experiments were planted earlier than standard blight fungicide trials (22 April 2015 in Ayrshire and 4 May 2016 for the two experiments in Ceredigion). Experiments were over-sprayed (including to 'untreated' plots) at 7 day intervals with a range of fungicides to prevent ingress of naturally occurring late blight inoculum prior to test treatments being applied. If fungicides were applied after plots were inoculated, the inoculated or infector plants were covered prior to fungicide sprays. Over-sprays were stopped at least 7 days prior to the inoculation date.

Table 1. *Combinations of cultivars and fungicide treatments tested in experiments in 2015 and 2016*

Treatment no.	Product applied	Rate applied [equivalent proportion of the maximum individual dose (where 1.0 = full dose) of metalaxyl-M in Fubol Gold]	Cultivar (foliar blight resistance rating)
1	Untreated	-	King Edward (3)
2	Untreated	-	Cara (5)
3	Ridomil Gold	0.051 [0.33]	King Edward (3)
4	Ridomil Gold	0.103 [0.67]	King Edward (3)
5	Ridomil Gold	0.051 [0.33]	Cara (5)
6	Ridomil Gold	0.103 [0.67]	Cara (5)

Production of inoculum and inoculation procedure for field experiments
Infector plants (cv. King Edward, grown in a polytunnel) were each inoculated with 5ml of a 1 x 10^5 sporangia/ml suspension where the concentration of 13_A2 and 6_A1 had been adjusted to a 5:95 ratio. Plants were maintained at high relative humidity for at least 48 hours to encourage infection. Once 'peppering' symptoms were observed, the plants were ready to be transplanted into plots. Two pot-sized holes were dug in the centre of each plot and a single infector plant was placed in each one.

Leaf sampling for genotyping
Leaf lesion samples for genotyping were taken when sporulating lesions were visible. Twenty-four leaves with sporulating lesions were randomly selected from the central two rows of each plot and incubated in Petri plates containing damp paper tissue prior to pressing onto FTA cards using the EuroBlight protocol method. Once dry, individual FTA cards were placed in separate small Ziploc plastic bags for transport to the James Hutton Institute for genotyping.

DISEASE ASSESSMENT AND EPIDEMIC PROGRESS

Foliar disease was assessed at least weekly during the season (as the percentage of leaf area affected) (Anon, 1976).

Selection coefficient and per capita growth rate

The rate of selection for 13_A2 was quantified for each treatment in each experiment as the selection coefficient (defined in van den Bosch *et al.*, 2014), calculated from the change in the proportion of 13_A2 in the population between inoculation and at least 7 days after the fungicide application). The *per capita* growth rate of the pathogen population (of all strains) was estimated, for each treatment and experiment, by fitting a logistic curve to the foliar late blight severity data. All analysis was done in Genstat 18th Edition (VSN International Ltd, UK).

RESULTS AND DISCUSSION

When comparing the epidemics within each of the experimental sites, the largest differences in foliar blight progress were between the two cultivars rather than fungicide treatments within cultivars (Figure 1). Comparing between sites, the epidemic was relatively slow at the Ayrshire site in 2015, with 58% leaf area affected by 31 August compared to 100% leaf area affected by the 13 August in Ceredigion. The epidemics at Ceredigion in 2015 and 2016 were similar in both years. The largest differences in *per capita* growth rate were between sites and seasons, rather than between treatments. Linear regression analysis showed that fitting separate lines for the two fungicide doses was not justified. Eighty-three percent of the variation was accounted for by regression lines, with a common intercept, fitted to the two cultivars with and without fungicide (Figure 2). Analysis is ongoing to test whether the regression model can be simplified further.

Carolan *et al.* (2017) predicted that any reduction in the growth rate of both the virulent and avirulent strains would decrease selection for virulence. The experimental data support this, with positive slopes in the fitted regression lines for all treatments. The variation in *per capita* growth rate arose predominantly from differences in environment between sites and seasons. Carolan *et al.* also demonstrated that, where virulence and fungicide insensitivity are not genetically linked, fungicide treatment would slow the selection for virulence, by reducing *per capita* growth rate. In the experimental system tested here, virulence and fungicide insensitivity appeared to be linked for 13_A2, demonstrated by the significantly greater slope for the fungicide treatment on Cara relative to the untreated. Hence, cultivar resistance and fungicide treatment slowed the epidemic, but also added to the selection pressure for 13_A2. Overall, selection for 13_A2 was not decreased by fungicide treatment in this scenario: the range of values for the selection coefficients for the more resistant cultivar (Cara) treated with fungicide and all King Edward treatments were similar. It appears that this was due to counteracting selection effects: cultivar resistance and fungicide treatment added to the selection pressure for 13_A2, but also reduced the *per capita* growth rate and therefore slowed the selection for virulence, together resulting in a neutral effect.

There is other evidence that more virulent strains may be selected for on resistant and partially resistant cultivars compared to more susceptible cultivars. When 13_A2 was first tested against cultivars, the foliar blight resistance rating for Cara was downgraded from a resistant (7) to moderately resistant (5) cultivar, whereas King Edward and other susceptible cultivars maintained their original rating (Lees *et al.*, 2012). Similarly, it has been demonstrated previously that 13_A2 was found at a higher proportion on partially resistant compared with susceptible cultivars and that very resistant cultivars, such Sarpo Mira and Bionica, select strongly for 13_A2 (Stellingwerf *et al.*, 2018). This provides strong evidence that where virulence and fungicide insensitivity are genetically linked in clonal variants, integrated control strategies need to consider both the impact of the strategy on the selection for virulence and the

effectiveness of the strategies in the field. Integrated disease management, particularly the use of cultivar resistance in combination with reduced fungicide input, has been proven to decrease the severity of foliar late blight (Fry, 1978; Kirk *et al.*, 2001; Kirk *et al.*, 2005; Nærstad *et al.*, 2007; Bain *et al.*, 2011). In these previous studies, the effectiveness of a cultivar/fungicide combination was tracked by assessing the progress of foliar blight. The impact of the strategies on the proportion of different *P. infestans* strains in these studies, was not determined.

Our findings suggest that where a clonal variant is able to overcome two or more control measures, then integrating those control measures is unlikely to slow selection for that variant. There are, however, scenarios in which integrated control could constrain pathogen evolution. Firstly, where sexual recombination predominates, for example *P. infestans* in Norway and Sweden, then the benefits of integration outlined in Carolan *et al.*, 2017 would apply. In that circumstance, it is still possible that a clonal variant with both host resistance and fungicide insensitivity will arise through sexual recombination and lead to a particularly fit clone, but integrated control may delay emergence of a fit clone. This could be important, as new clones of *P. infestans* have been detected initially in predominately sexual populations (e.g. 13_A2) and have gone on to spread across Europe. Secondly, using a cultural control method that slows the epidemic growth rate, but does not cause selection pressure, should slow selection for both virulence and insensitivity.

As legislation moves towards a hazard based rather than risk based assessment for pesticides, and plant pathogens shift towards more virulent strains and fungicide insensitivity, it is necessary to implement strategies that will be sustainable and cost effective. There is therefore a need to consider both the effectiveness of integrated strategies on disease control, but also the impact of such strategies and legislation on the evolution of future pathogen populations and our ability to maintain control.

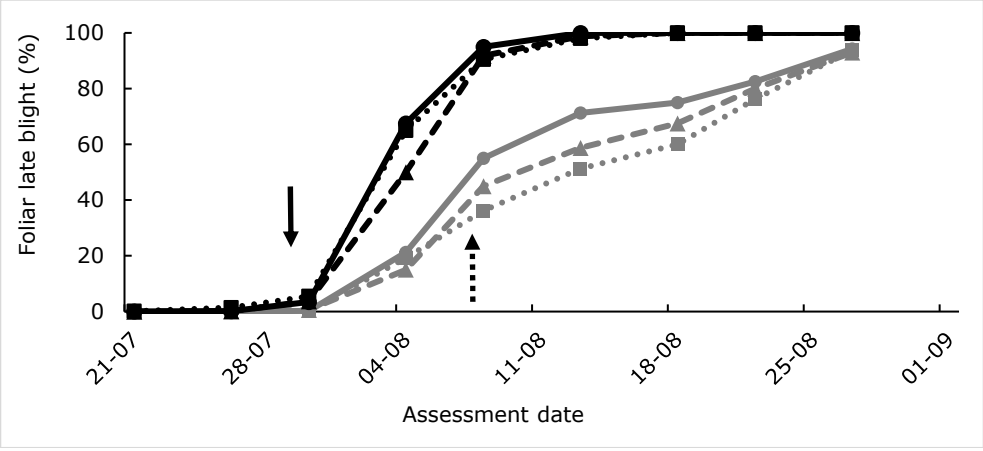

Figure 1. *Disease progress for fungicide dose and cultivar combinations at Ceredigion in 2016. King Edward (black) and Cara (grey). No fungicide (circles), $^1/_3$ dose (triangles) and $^2/_3$ dose (squares). Arrows represent when the fungicide was applied (solid arrow: 30 July) and when the lesion samples were taken (dotted arrow: 8 August)*

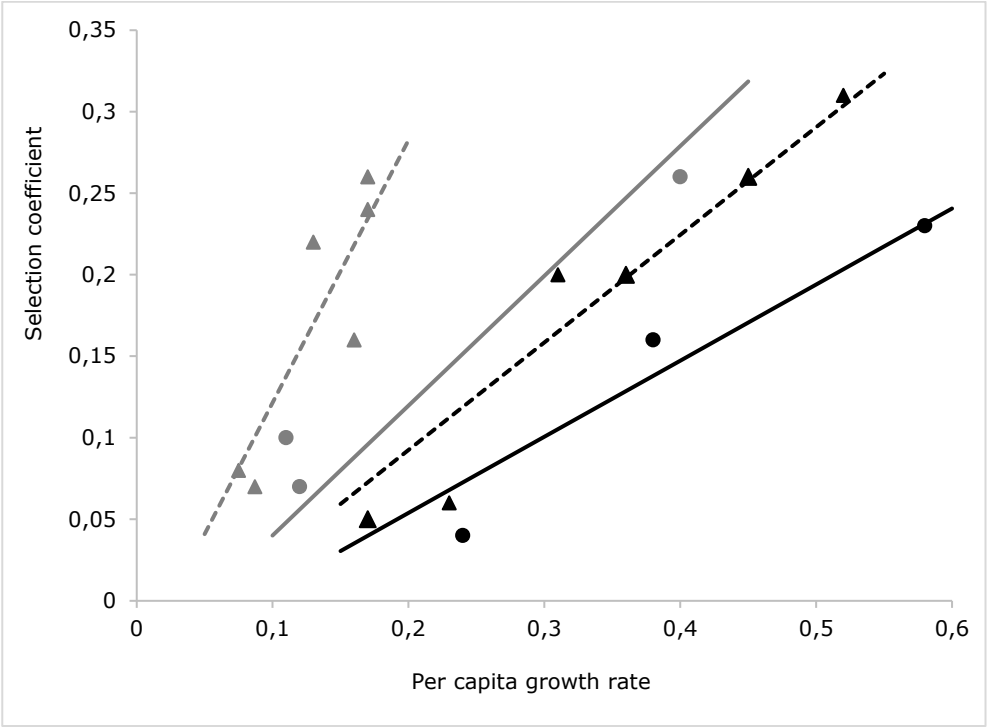

Figure 2. *Selection coefficient plotted on per capita growth rate of the epidemic for each fungicide dose and cultivar combination from three field experiments (R^2 = 0.83). King Edward (black) and Cara (grey). No fungicide (circles) and with fungicide (triangle). The lines represent the fitted model for with (dotted lines) and without (solid lines) fungicide for each cultivar. The difference between fungicide doses did not justify separate regression lines*

ACKNOWLEDGEMENTS

The trials were part of a Horticulture and Potato Initiative (HAPI) project funded by BBSRC, AHDB Potatoes, BASF, Belchim and Syngenta Crop Protection. The isolates of genotype 6_A1 and 13_A2 used to inoculate the trials were kindly provided to ADAS and SRUC by The James Hutton Institute.

REFERENCES

Anon (1976). Manual of Plant Growth Stages and Disease Assessment Keys. MAFF Publications, Pinner, Middlesex.

Bain, R.A., Ritchie, F., Lees, A., Dyer, C., and Roberts, A. (2011). Development of foliar late blight (*Phytophthora infestans*) in relation to cultivar resistance and fungicide dose on potato. Proceedings of the thirteenth workshop of a European network for development of an integrated control strategy of potato late blight. In: H.T.A.M Schepers (ed.). PPO Special Report no. 15, 139-144.

British Potato Cultivar Database. http://cultivars.potato.org.uk/menu.php Accessed 11 October 2019.

Carolan, K., J. Helps, F. van den Berg, R. Bain, N. Paveley and F. van den Bosch, (2017). Extending the durability of cultivar resistance by limiting epidemic growth rates. Proceedings of the Royal Society B: Biological Sciences 284 (1863): 20170828.

Carter, G.A., R.M. Smith and K.J. Brent (1982). Sensitivity to metalaxyl of *Phytophthora infestans* populations in potato crops in south-west England in 1980 and 1981. Annals of Applied Biology 100, 443-441.

Fry, W.E. (1978). Quantification of general resistance of potato cultivars and fungicide effects for integrated control of late blight. Phytopathology 68, 1650-1655.

Holmes, S.J.J., and A.G. Channon (1984). Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. Plant Pathology 33, 347-354.

Kirk, W.W., K.J. Felcher, D.S. Douches and J. Coombs (2001). Effect of host plant resistance and reduced rates and frequencies of fungicide application to control potato late blight. Plant Disease 85, 1113 – 1118.

Kirk, W.W., F.M. Abu-El Samen, J.B. Muhinyuza, R. Hammerschmidt, D.S. Douches, C.A. Thill, H. Groza and A.L. Thompson (2005). Evaluation of potato late blight management utilising host plant resistance and reduced rates and frequencies of fungicide applications. Crop Protection 24, 961-970.

Lees, A.K., J.A. Stewart, J.S. Lynott, S.F. Carnegie, H. Campbell, A.M.I. Roberts (2012). The effect of a dominant *Phytophthora infestans* genotype (13_A2) in Great Britain on host resistance to foliar late blight in commercial potato cultivars. Potato Research 55, 125-134.

Naerstad, R., A. Hermansen and T. Bjor (2007). Exploiting host resistance to reduce the use of fungicides to control potato late blight. Plant Pathology 56, 156-166.

Schepers, H.T.A.M., G.J.T. Kessel, F. Lucca, M.G. Förch, G.B.M. van den Bosch, C.G. Topper, A. Evenhuis (2018). Reduced efficacy of fluazinam, against *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 151, 947-960.

Stellingwerf, J.S., S. Phelan, F.M. Doohan, V. Ortiz, D. Griffin, A. Bourke, R.C.B. Hutten, D.E.L. Cooke, S. Kildea, E. Mullins (2018). Evidence for selection pressure from resistant potato genotypes but not from fungicide application within a clonal *Phytophthora infestans* population. Plant Pathology 67, 1528-1538.

van den Bosch, F., R. Oliver, F. van den Berg, N. Paveley (2014). Governing principles can guide fungicide resistance management tactics. Annual Review of Phytopathology 52, 175-195.