Received: 11 January 2021 | Accepted: 16 July 2021

DOI: 10.1111/1365-2664.13978

RESEARCH ARTICLE

Journal of Applied Ecology | BRITISH ECOLOGICAL SOCIETY

Plant diversity ameliorates the evolutionary development of fungicide resistance in an agricultural ecosystem

Li-Na Yang[1] | Oswald Nkurikiyimfura[2] | Zhe-Chao Pan[3] | Yan-Ping Wang[2] | Abdul Waheed[2] | Ruey-Shyang Chen[4] | Jeremy J. Burdon[5] | Qi-Jun Sui[3] | Jiasui Zhan[6]

[1]Institute of Oceanography, Minjiang University Fuzhou, China; [2]Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, China; [3]Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China; [4]Department of Biochemical Science & Technology, National Chiayi University, Chiayi, Taiwan; [5]Plant Industry, CSIRO, Canberra, ACT, Australia and [6]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence
Jiasui Zhan, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
Email: Jiasui.zhan@slu.se

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31460368 and 31901861

Handling Editor: Maria Fernanda Peñaflor

Abstract

1. The evolution of fungicide resistance in agricultural and natural ecosystems is associated with the biology of pathogens, the chemical property and application strategies of the fungicides. The influence of ecological factors such as host diversity on the evolution of fungicide resistance has been largely overlooked but is highly relevant to social and natural sustainability. In this study, we used an experimental evolution approach to understand how host population heterogeneity may affect the evolution of fungicide resistance in the associated pathogens.
2. Potato populations with six levels of genetic heterogeneity were grown in the same field and naturally infected by *Phytophthora infestans*. Pathogen isolates (~1,200) recovered from the field experiment were molecularly genotyped. Genetically distinct isolates were selected from each population and 142 isolates were assayed for their tolerance to two fungicides differing in the mode of action. Tolerance was determined by calculating the relative growth rate of the isolates in the presence and absence of fungicides and the effective concentration for 50% inhibition.
3. The evolution of fungicide resistance in *P. infestans* was affected by the genetic variation of host populations. Higher potato diversification increased the sensitivity of *P. infestans* to both fungicides and reduced genetic variation of the pathogen available for the development of fungicide resistance. These mitigating effects are independent of biochemical properties of fungicides and are likely caused by host selection for pathogen strains differing in the ability of fungicide influxes, effluxes or detoxification rather than mutations in fungicide target genes.
4. *Synthesis and applications*. The development of fungicide resistance greatly threatens food security and ecological sustainability, and it is urgent need to develop

Li-Na Yang and Oswald Nkurikiyimfura contributed equally to the manuscript.

agricultural practices which can ameliorate this problem. Our results show that potato crop with a higher genetic diversity is associated with a late blight pathogen of higher fungicide sensitivity and lower potential of developing fungicide resistance, indicating that agricultural diversification such as through cultivar mixture can reduce the application dose and frequency of fungicides needed to achieve the same level of disease control, which, in turn, further reduce the selection pressure acting on the pathogen populations and the evolutionary risk of developing fungicide resistance in pathogens. Together with benefits documented in other studies, our results indicate that crop diversification is an eco-friendly approach that not only ameliorate fungicide resistance but also help achieve social and ecological sustainability by balancing the interaction among food security, socio-economic development and ecological resilience and should be promoted.

KEYWORDS

biodiversity, disease management, fungicide resistance, mode of action, natural selection, *Phytophthora infestans*, plant pathogens, sustainability

1 | INTRODUCTION

Resilient agriculture seeks to increase the production of high-quality foods to meet immediate social and economic requirements while simultaneously maintaining healthy ecosystems to support the future needs of society. Fungicide resistance is considered as a major challenge to agricultural sustainability, and an important and intrinsic component of social and natural sustainability (Fisher et al., 2018; Hahn, 2014). Plant diseases caused by microbes greatly threaten global food production and socio-economic development, causing annual yield reductions of 13%–22% in major food crops and concomitant billions of dollars of economic loss (Burdon et al., 2020). Fungicides are among the main approaches to battle fungal and oomycete diseases. However, the development of fungicide resistance increases the risk of plant disease epidemics, greatly threatening food security and sustainability. In practice, such reductions in fungicide efficacy are usually countered by either increasing application doses and frequency, or by developing new fungicides, increasing economic costs of production and the potential for food contamination. Increased fungicide application may also elevate damage to biodiversity and impede ecological resilience in farming and adjacent areas (Fisher et al., 2018), thereby generating longer term negative impacts on future agricultural productivity and economic development.

The evolution of fungicide resistance can arise from mutations and/or altered expression of target genes. Biology and physiology of pathogens are the main factors that affect the mutation, expression and fitness of target genes and, therefore, influence the evolutionary potential of fungicide resistance (Grimmer et al., 2015). For example, pathogens with short generation times have a higher potential to develop fungicide resistance as short generation times ensure a greater chance of mutations within a given timescale (Stecher et al., 2013; Zhan et al., 2002). Similarly, pathogens producing large number of sexual spores that travel long distances are also more likely to develop fungicide resistance due to their enhanced genetic variation and ability to spread novel mutants widely (Zhan et al., 2001, 2014).

The chemical property of effective compounds, that is, their mode of action, is another factor determining the evolutionary risk of fungicide resistance in pathogens (Hawkins & Fraaije, 2018; Jørgensen et al., 2017; Lucas et al., 2015). Fungicides can be divided into site-specific and site-non-specific groups based on their chemical properties. Site-specific fungicides disrupt key cellular processes of pathogens by binding their effective compounds to specific protein targets. Resistance to these fungicides arises relatively easily as it usually emerges due to mutation and enhanced expression of a single gene (Lucas et al., 2015). In contrast, site-non-specific fungicides act on a range of cellular processes that impact collectively on the fitness of pathogens. Resistance to these fungicides results from a series of changes in many genes in pathogen genomes, and is therefore less likely to occur (Hawkins & Fraaije, 2018; Lucas et al., 2015).

Disease expression, epidemics and subsequent responses to fungicide management result from an ongoing interaction among pathogens, hosts and environments in an ecological framework. In addition to pathogen biology and fungicide properties, environmental factors may also contribute to the development of fungicide resistance in pathogens (He et al., 2018; Lurwanu et al., 2020). Pathogen biology and fungicide properties mainly affect the appearance of new mutants conferring fungicide resistance. In contrast, environmental conditions not only affect the generation of resistant mutants through their impact on pathogen life cycles and fungicide toxicity, but also affect the maintenance of mutants in populations through influences on the type and intensity of selection (Mitchell et al., 2005). For example, it has been documented that environmental homogeneity poses directional selection on pathogens, facilitating the development of fungicide resistance while disruptive

selection created by environmental heterogeneity reduces the evolution of fungicide resistance (Rex Consortium, 2013).

Surprisingly, to date, studies of environmental impacts have been focused on the contribution of abiotic factors, in particular, application strategies of fungicides such as spatiotemporal variation in fungicide dose, frequency or compounds (Jørgensen et al., 2017; van den Bosch et al., 2014). In contrast, the influence of ecological factors such as host genetic diversity on the evolution of fungicide resistance has been largely overlooked. Host population heterogeneity may benefit agricultural ecosystems in a diversity of ways: increasing crop yield and resilience, reducing disease epidemics, mitigating pathogen dynamics and improving soil health (Creissen et al., 2016; Yang et al., 2019; Zhu et al., 2000). However, it is not clear whether, and to what extent, host population heterogeneity may affect the development of fungicide resistance in pathogens.

In this study, we used the potato (*Solanum tuberosum* L.)–*Phytophthora infestans* interaction to study the impact of host heterogeneity on the evolution of fungicide resistance. In particular, we tested whether host diversity serves as a selection agent driving the development of fungicide resistance in *P. infestans*. Potato is the world's third largest food crop, contributing greatly to global food security and socio-economic development. *Phytophthora infestans* is the most destructive pathogen of potato and a major yield-limiting factor. Whole potato fields can be destroyed by the pathogen within a few days under favourable climatic conditions. The disease causes up to a 70% yield reduction, and annual economic losses has been estimated to be >$3 billion dollars world-wide (Fontem & Aighewi, 1993; Fry, 2008). Currently, late blight disease is mainly suppressed by frequent fungicide applications (Kirk et al., 2001). Continuous and intensive fungicide applications pose strong selection on the pathogen, resulting in a rapid development of fungicide resistance (Lucas et al., 2015). In turn, the typical response of increasing application concentration and frequency to counter declining efficacy escalates the economic burden on farmers and creates negative effects on human health and agricultural resilience. There is, therefore, an urgent need to understand the eco-evolutionary mechanisms of fungicide resistance and use that knowledge to develop mitigating approaches.

To achieve these goals, we grew 31 potato populations varying in genetic heterogeneity by planting six cultivars individually (monocultures) or in combinations of an equal proportion (mixture) of two to six cultivars in the field (Yang et al., 2019). The host populations were challenged by *P. infestans* naturally, and pathogen populations were collected during the epidemics. Tolerance to the fungicides mancozeb and azoxystrobin was tested under laboratory conditions by measuring, and comparing, the relative growth rate (RGR) of the pathogen in the presence and the absence of the fungicides and the effective concentration for 50% inhibition (EC50) values. The two fungicides differ in mobility, function and mode of action. Mancozeb is a site-non-specific, multi-target contact fungicide that acts on plant surfaces and prevents the invasion of pathogens into host cells (Gullino et al., 2010), while azoxystrobin is a site-specific, single target fungicide that penetrates host cells and kills pathogens inside (Bartlett et al., 2002).

2 | MATERIALS AND METHODS

2.1 | *Phytophthora infestans* populations and their genotyping

Phytophthora infestans populations used in the study originated from a field experiment aimed at understanding the ecological, evolutionary and productive roles of biodiversity (Yang et al., 2019). The field experiment was conducted in 2012 in Yema, Yunnan, southwest China (26.10°N, 103.38°E)—one of the largest potato production regions in the country. Yunnan also represents one of the most diverse ecosystems in China and has environmental conditions favourable for late blight epidemic. Consequently, the region routinely experiences severe late blight epidemics and is known to have the most complex population structure and fastest evolution of *P. infestans* in China (Wu et al., 2016). Detailed field experimental design and collection and molecular genotyping of the pathogen can be found in the previous publication (Yang et al., 2019). Briefly, six potato cultivars varying in agronomic traits and quantitative *P. infestans* resistance were grown alone (monoculture) or in random mixtures of equal proportions of two, three, four, five or six cultivars, generating six potato diversity levels in total. When grown in the field, all six cultivars were infected by the local *P. infestans* population with levels of disease in the six cultivars ranged from 63 to 2,396 in AUDPC (area under the disease progress curve; Yang et al., 2019).

The experiment contained 31 host treatments (populations) with six host treatments in each of the monoculture, two-, three-, four- and five-cultivar mixtures and one host treatment in the six-cultivar mixture (Table S1). The experimental treatments were laid out in a randomized complete block design with three replicates. Each host treatment was grown in a ~25-m^2 plot. The potato treatments were naturally infected by *P. infestans* spreading from the local surroundings, and no fungicides were applied during the whole potato growing season. To avoid the potential bias being introduced by a particular cultivar in the measurement of fungicide resistance, the 31 host treatments were constructed in such a way that each cultivar was equally represented in each of the six diversity groups (Table S1).

In the middle of epidemics, potato leaves infected with *P. infestans* were collected from all experimental treatments. Approximately 20 infected leaves were collected from the inner rows of each plot with only one leaf sampled from any given plant. Each sampled leaf was placed separately into a sandwich bag to prevent cross-contamination and taken to the laboratory for pathogen isolation and purification. Only one single *P. infestans* strain was isolated from each leaf with ~1,200 *P. infestans* isolates being collected from the entire experiment. The *P. infestans* isolates were grouped into six populations each corresponding to one of the six

host diversity levels by combining isolates from the same host diversity level. For example, isolates from all six monoculture cultivars were grouped together into a *P. infestans* population with the host diversity level of 1. Similarly, isolates from all six two-cultivar mixtures were grouped together into a *P. infestans* population with the host diversity level of 2 and so on. Genotypes of the *P. infestans* isolates were determined by molecular amplification of genomic DNA with SSR markers, restriction enzyme-PCR amplification of mitochondrial haplotypes, mating type and sequence analysis of Avr3a and Avripio effector genes (Yang et al., 2019; Zhu et al., 2015). Isolates differing in any of these markers were considered as distinct genotypes.

2.2 | Fungicide tolerance measurement

To rule out potential over-representation of genotypes from clonal reproduction, only *P. infestans* isolates with distinct genotypes were selected and tested for fungicide tolerance. Same dominant genotypes were detected in *P. infestans* populations from the six cultivars grown in monoculture or from different mixtures, and no evidence for isolate–cultivar association was found (Yang et al., 2019). When multiple isolates of a particular genotype were detected in a *P. infestans* population, only one of them was included for fungicide test. Prior to the fungicide test, isolates maintained at long-term storage were revived on rye B agar at 18°C for 8 days. Mycelial plugs ($\phi = 5$ mm) taken from the margins of revived colonies were transferred to fresh 9-cm rye B agar plates either amended with four concentrations (5, 10, 15 and 20 μg/ml) of mancozeb, four concentrations (0.01, 0.05, 0.1 and 0.15 μg/ml) of azoxystrobin or without the supplementation of the fungicides (controls). These were then cultured at 18°C in the dark for 8 days. All fungicide treatments and controls were replicated three times, and all plates were laid out in a completely randomized design in incubators. Colonies were photographed daily between 2 and 8 days post-inoculation and their areas were measured using Assess (Lamari, 2002). A total of ~50,000 [142 isolates × 2 fungicides × 3 replicates × 8 treatments (4 concentrations + 4 controls) × 7 measures] data were collected from the fungicide tests.

2.3 | Statistical analysis

The growth rate of isolates was estimated using a logistic model (Aguayo et al., 2014) based on colony sizes quantified at each time point over the 8-day inoculation period under each fungicide concentration and control. The initial colony size at the inoculation day was set to 0.2 cm^2 ($\pi r^2 = 3.14 \times 0.25^2$), and the capacity of colony growth (K) for the logistic model was set to 63.59 cm^2 ($\pi r^2 = 3.14 \times 4.5^2$, here 4.5 is the radius of Petri dishes). Pathogen tolerance to mancozeb and azoxystrobin was measured by RGR and EC50 (Brunner et al., 2016; Zhan et al., 2006). The mean relative growth rate (MRGR) of isolates was calculated by taking the average of the RGRs at the four fungicide concentrations. The analyses of variance for fungicide tolerances were performed using the general linear model procedure of SAS where 'concentration' was treated as a fixed variable and 'isolate (host diversity)' and 'host diversity' as random variables. Least significant difference (LSD) (Kokalis-Burelle et al., 2013) was used to compare fungicide tolerance among the six host diversity levels of *P. infestans* populations.

SSR data of the isolates were taken from a previous publication (Yang et al., 2019), and population differentiation in SSR loci among the pathogens from different host diversity levels was estimated by G_{ST} (Nei, 1987) using Popgene 3.2 (Yang et al., 1996). Phenotypic variance in RGR was partitioned into sources attributable to differences among host diversity, among isolates within host diversity, fungicide concentration and isolate–concentration interaction using SAS GLM and VARCOMP programs (SAS 9.3 Institute) as described previously (Yang et al., 2016). Population differentiation (Q_{ST}) in RGRs among pathogen isolates from different host diversity levels was estimated in a way like G_{ST} by calculating the proportion of total genetic variation attributable to among-population variation as follows:

$$Q_{ST} = \frac{\delta^2_{AP}}{\delta^2_{AP} + \delta^2_{WP}},$$

where δ^2_{AP} and δ^2_{WP} represent the genetic variance in RGR attributed to among-population (host diversity) and within-population variation respectively. The population differentiation was estimated by calculating both pairwise (considering only two diversity levels each time) and overall (considering all six diversity levels together at one time) Q_{ST} and G_{ST}. The overall Q_{ST} and G_{ST} were compared by *t*-tests using standard deviations generated from 100 bootstrapping resamples of the original RGRs as described previously (Zhan & McDonald, 2011).

The genetic and epigenetic effects of host diversity on fungicide tolerance were measured by heritability and phenotypic plasticity. The heritability of RGR in populations was estimated by dividing genetic variance within the population by total phenotypic variance. The phenotypic plasticity of RGR was calculated by dividing the variance of isolate–concentration interaction by total phenotypic variance (Tonsor et al., 2013). Associations among fungicide tolerances, population differentiation and host diversity levels were evaluated by Pearson correlation (Ott, 1992).

3 | RESULTS

3.1 | Frequency distribution of fungicide tolerance in *Phytophthora infestans* populations from different host diversity levels

RGRs of both fungicides displayed a continuous and unimodal distribution with right shifts as the host diversity level decreased when the *P. infestans* populations from different host diversity levels were considered individually (Figure 1a,b). These patterns were consistent across all fungicide concentrations (Figures S1 and S2). Combined

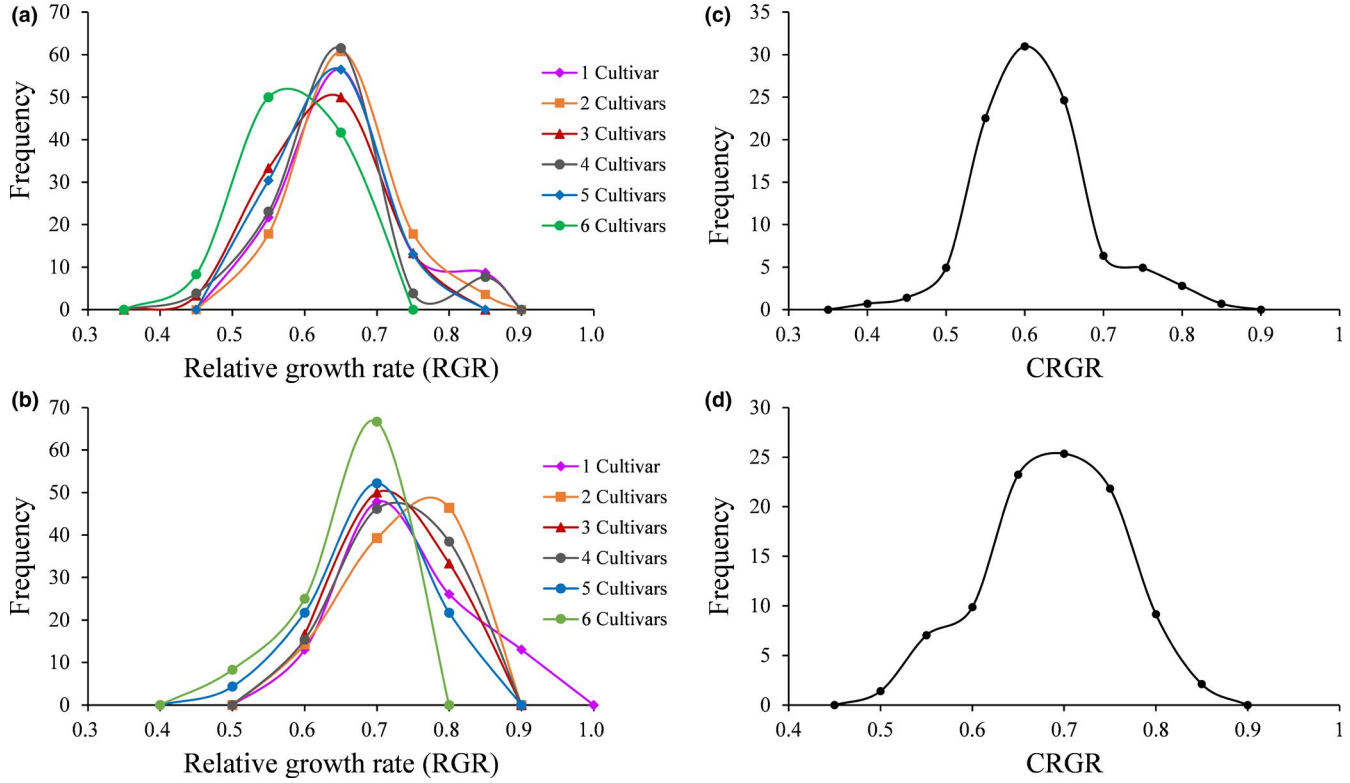


FIGURE 1 Frequency distribution of mancozeb and azoxystrobin tolerance in the *Phytophthora infestans* populations collected from different host diversity: (a) relative growth rate (RGR) on mancozeb in each *P. infestans* population; (b) RGR on azoxystrobin in each *P. infestans* populations; (c) combined RGR (CRGR) for mancozeb when *P. infestans* isolates from different populations were pooled; and (d) combined RGR (CRGR) for azoxystrobin when *P. infestans* isolates from different populations were pooled

RGRs also displayed a continuous and unimodal distribution when the *P. infestans* populations from different host diversity levels were pooled (Figure 1c,d). The combined RGRs ranged from 0.38 to 0.81 with a mean of 0.59 and from 0.50 to 0.84 with a mean of 0.66 for mancozeb and azoxystrobin respectively (Figure 1).

EC50 values ranged from 6.60 to 20.65 μg/ml with a mean of 10.22 μg/ml for mancozeb and from 0.05 to 0.22 μg/ml with a mean of 0.12 μg/ml for azoxystrobin (Figure 2). Consistent with RGRs, EC50 for mancozeb and azoxystrobin also displayed a continuous and unimodal distribution regardless of whether the *P. infestans* populations were considered individually according to each host diversity level or combined. For mancozeb EC50, the same pattern was observed among the pathogen populations from different host diversity levels with a more right-spread distribution towards populations taken from lower diversity host populations (Figure 2a). For azoxystrobin EC50, the frequency distribution gradually shifted to the right as host population diversity decreased (Figure 2b).

3.2 | Fungicide tolerance significantly differed among *P. infestans* populations from different host diversity levels

Host diversity, isolate, concentration and/or isolate–concentration interactions all significantly affected *P. infestans* tolerance to mancozeb and azoxystrobin measured with RGR and EC50 ($p < 0.0001$; Table S2). In all cases, *P. infestans* populations from host diversity 5 and 6 displayed significantly lower mancozeb and azoxystrobin tolerance (lower RGR and EC50) than those from host diversity 1 and 2 (Table 1). In most cases, *P. infestans* populations from host diversity 3 and 4 displayed significantly lower mancozeb and azoxystrobin tolerance than those from host diversity 1 and 2, but significantly higher mancozeb and azoxystrobin tolerance than those from host diversity 5 and 6. These association patterns were consistent across all individual concentrations of the two fungicides.

3.3 | Natural selection driven by host population diversity rather than genetic drift is the primary mechanism for *P. infestans* divergence in fungicide tolerance

Evolutionary mechanisms responsible for the difference in fungicide tolerance among *P. infestans* populations can be determined through the comparative analysis of genetic differentiation in fungicide resistance (Q_{ST}) and in neutral SSR markers (G_{ST}). Significant difference between Q_{ST} and G_{ST} would suggest that the population polymorphism in fungicide resistance resulted from natural selection driven by host population diversity while similar Q_{ST} and G_{ST}

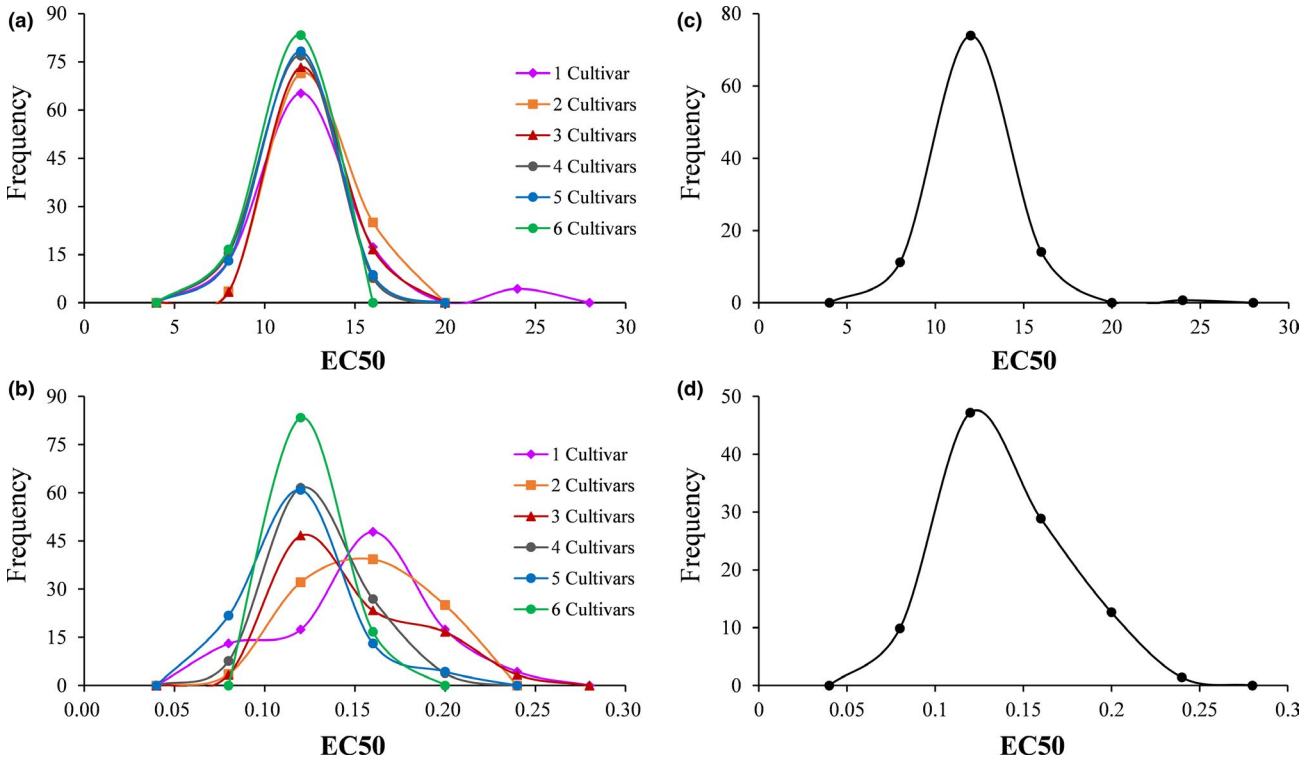


FIGURE 2 Frequency distribution of mancozeb and azoxystrobin tolerance determined by EC50 in the *Phytophthora infestans* populations collected from different host populations diversity: (a) mancozeb in each *P. infestans* population; (b) azoxystrobin in each *P. infestans* population; (c) combined mancozeb EC50 when *P. infestans* isolates from different populations were pooled; and (d) combined azoxystrobin EC50 when *P. infestans* isolates from different populations were pooled

values would suggest that the difference in fungicide resistance among *P. infestans* populations was generated by genetic drift. The pairwise G_{ST} across the SSR marker loci ranged from 0.00 to 0.10 with an average of 0.03, while the pairwise Q_{ST} for mancozeb and azoxystrobin tolerance ranged from 0.00 to 0.31 with an average of 0.08 and from 0.00 to 0.65 with an average of 0.22 (Table S3) respectively. Pairwise Q_{ST} in mancozeb and azoxystrobin tolerances was positively associated with each other and linearly increased with the increasing difference in host diversity level (Figure 3). Pairwise G_{ST} did not correlate with difference in host diversity (Figure 3). The overall Q_{ST} in mancozeb and azoxystrobin tolerance was 0.05 and 0.25, respectively, which was significantly higher than the overall G_{ST} (0.03) when the *P. infestans* isolates from the six host diversity levels were combined.

3.4 | Host genetic diversity negatively affects *P. infestans* tolerance to fungicides

Both RGR and EC50 of the fungicides were negatively associated with host diversity level regardless of whether the analysis was performed on individual fungicide concentrations (Figures S3 and S4) or pooled across all concentrations (Figure 4). Host diversity level was also negatively associated with the phenotypic variance of *P. infestans* tolerance to mancozeb measured by either RGR or EC50 (Figure 5a, c). The negative relationship with respect to *P. infestans* tolerance to azoxystrobin was significant for the EC50 assessment (Figure 5d), but not significant with respect to variance in RGR for azoxystrobin (Figure 5b).

3.5 | Epigenetics plays a more important role than genetics in fungicide tolerance of *P. infestans*

The heritability of mancozeb tolerance ranged from 0.022 to 0.054 with a mean of 0.036 while plasticity ranged from 0.053 to 0.076 with a mean of 0.064 (Table 2). The plasticity was 1.19–3.46 (mean = 1.79) fold higher than the heritability. For azoxystrobin tolerance, heritability ranged from 0.010 to 0.052 with a mean of 0.026 while plasticity ranged from 0.087 to 0.148 with a mean of 0.115. The plasticity was 1.65–8.82 (mean = 4.39) fold higher than the heritability (Table 2). The plasticity and heritability ratio tended to increase as host diversity level increased for both mancozeb and azoxystrobin, but none of the associations were significant ($r = 0.58$, 0.16 and $p = 0.2276$, 0.7475 respectively).

3.6 | Concordant impact of host diversity on *P. infestans* tolerance to fungicides with different chemical properties

Mancozeb is a multi-target contact fungicide while azoxystrobin is a site-specific systemic fungicide. *Phytophthora infestans* tolerances

TABLE 1 Least significant difference test for the relative growth rate (RGR) and the effective concentration of 50% inhibition (EC50) of the six *Phytophthora infestans* populations collected from different potato diversity. Values followed by different letters in the same column differ significantly at $p = 0.05$

	Mancozeb							Azoxystrobin						
	RGR (cm^2/day)					EC50		RGR (cm^2/day)					EC50	
	5[1]	10	15	20				0.01	0.05	0.1	0.15			
Diversity	(μg/ml)				Mean	Value	Ratio[2]	(μg/ml)				Mean	Value	Ratio
1	0.916^{A}	0.706^{A}	0.525^{A}	0.297^{A}	0.611^{A}	10.531^{B}	14.049	0.958^{A}	0.854^{A}	0.603^{A}	0.370^{A}	0.697^{A}	0.134^{A}	0.157
	(0.020)[3]	−0.027	−0.027	−0.022	−0.019	−0.669		−0.017	−0.028	−0.036	−0.027	−0.02	−0.009	
2	0.914^{A}	0.712^{A}	0.507^{A}	0.280^{B}	0.603^{A}	10.946^{A}	7.721	0.949^{B}	0.841^{A}	0.587^{B}	0.350^{B}	0.682^{AB}	0.133^{A}	0.118
	−0.018	−0.016	−0.028	−0.016	−0.015	−0.415		−0.014	−0.022	−0.026	−0.023	−0.012	−0.007	
3	0.915^{A}	0.695^{B}	0.495^{B}	0.255^{C}	0.590^{B}	10.192^{C}	6.657	0.931^{CD}	0.782^{B}	0.579^{B}	0.367^{B}	0.665^{C}	0.120^{B}	0.154
	−0.038	−0.024	−0.026	−0.019	−0.015	−0.342		−0.016	−0.026	−0.026	−0.026	−0.014	−0.008	
4	0.888^{B}	0.704^{A}	0.511^{B}	0.254^{C}	0.589^{B}	9.668^{D}	6.211	0.94°C	0.793^{B}	0.578^{B}	0.352^{B}	0.666^{BC}	0.113^{C}	0.089
	−0.019	−0.024	−0.031	−0.019	−0.018	−0.364		−0.018	−0.029	−0.031	−0.034	−0.016	−0.005	
5	0.885^{B}	0.682^{B}	0.482^{B}	0.222^{E}	0.568^{C}	10.139^{C}	5.088	0.900^{D}	0.769^{C}	0.577^{C}	0.279^{C}	0.631^{D}	0.103^{D}	0.109
	−0.014	−0.024	−0.032	−0.011	−0.015	−0.361		−0.022	−0.031	−0.034	−0.026	−0.019	0.007)	
6	0.883^{C}	0.645^{C}	0.431^{C}	0.236^{D}	0.549^{D}	9.372^{E}	4.797	0.890^{E}	0.699^{D}	0.557^{C}	0.30°C	0.611^{E}	0.104^{D}	0.042
	−0.024	−0.038	−0.031	−0.024	0.020)	−0.499		−0.031	−0.049	−0.05	−0.03	−0.024	−0.005	
Average	0.9	0.691	0.492	0.257	0.585	10.141	14.049	0.928	0.79	0.58	0.336	0.658	0.118	0.157
	−0.008	−0.01	−0.012	−0.008	−0.007	−0.185		−0.008	−0.012	−0.013	−0.012	−0.007	−0.003	

[1]Fungicide concentration.

[2]Ratio between highest and lowest EC50.

[3]95% confidence interval.

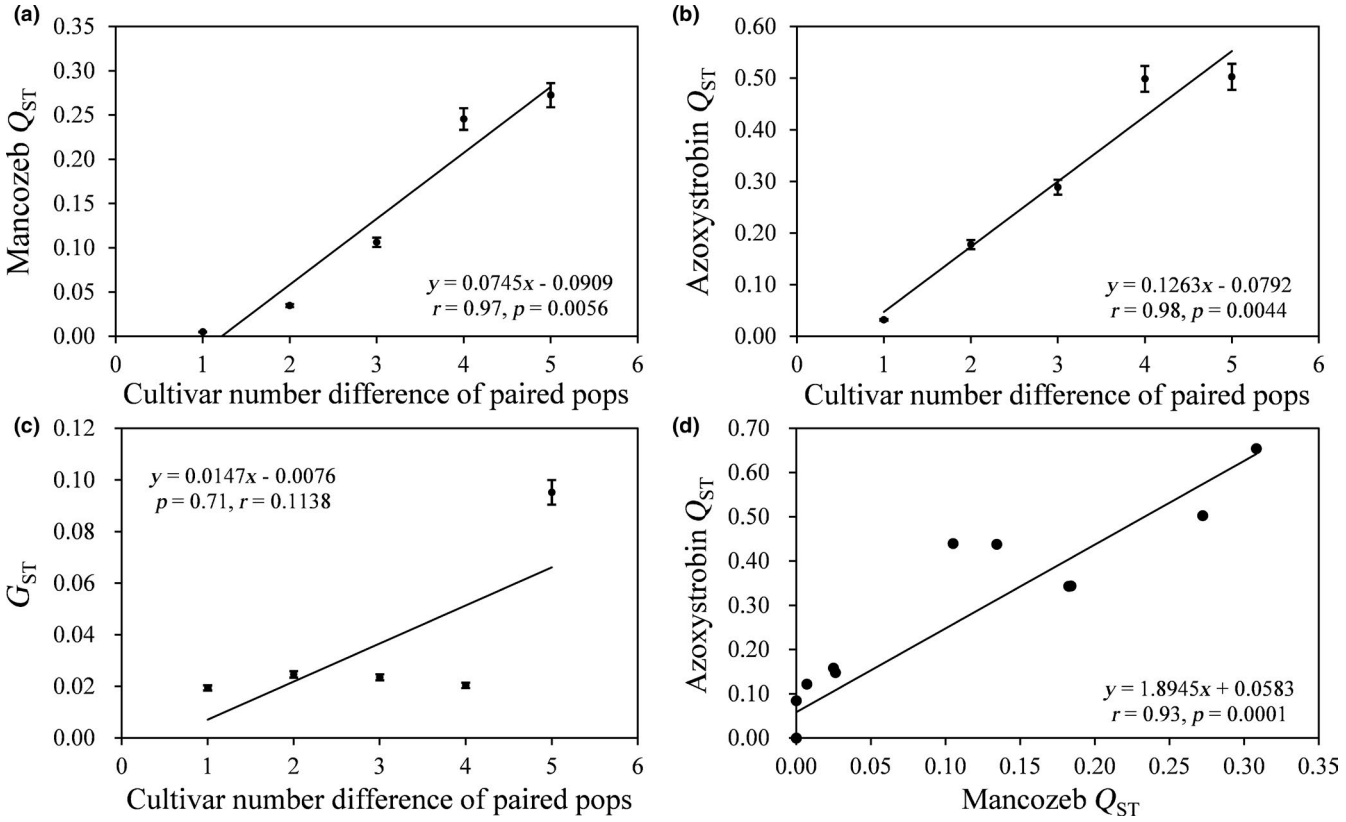
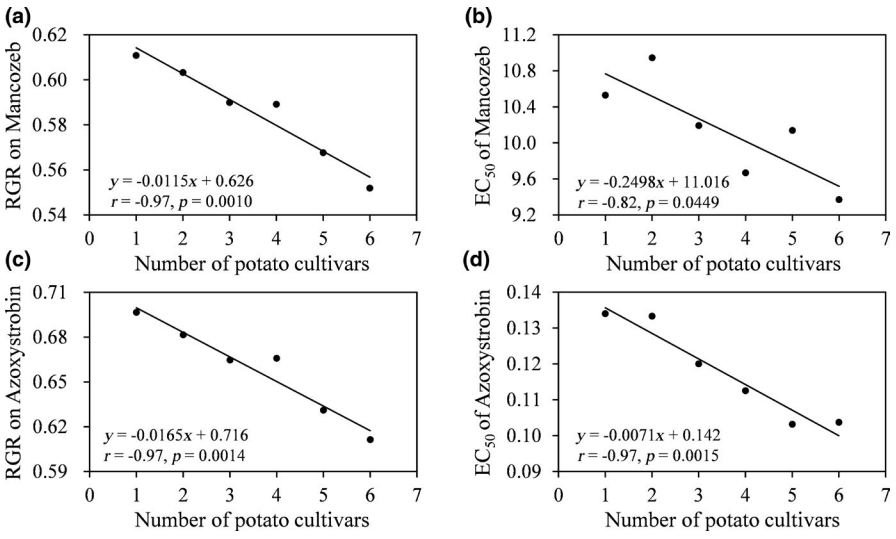


FIGURE 3 Impact of host population diversity on pairwise population differentiation of *Phytophthora infestans*: (a) Q_{ST} in mancozeb tolerance; (b) Q_{ST} in azoxystrobin tolerance; (c) G_{ST} in neutral SSR markers; and (d) correlation between Q_{ST} of mancozeb and azoxystrobin tolerance

FIGURE 4 Impact of host population diversity on fungicide tolerance: (a) relative growth rate (RGR) on mancozeb; (b) EC50 of mancozeb; (c) RGR on azoxystrobin; and (d) EC50 of azoxystrobin



to mancozeb and azoxystrobin measured by either RGR or EC50 were positively correlated regardless of whether the association was evaluated at the population level (Figure 6a,b) or at the individual isolate level (Figure 6c,d). This result indicates that the negative impact of host genetic diversity on the development of fungicide resistance in *P. infestans* was not affected by the mode of action of the fungicides.

4 | DISCUSSION

We found that *P. infestans* populations collected from different potato diversity levels varied significantly in mancozeb and azoxystrobin tolerance (Table 1; Table S2). Given the randomized experimental design, the use of locally certified clean seed and the collection of pathogen isolates at the same time point of the

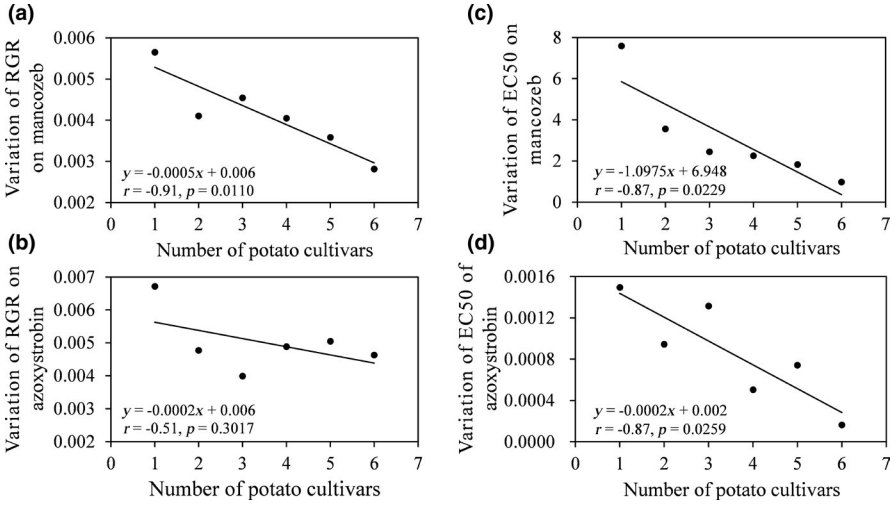


FIGURE 5 Impact of host diversity on the variation of fungicide tolerance in *Phytophthora infestans*: (a) RGR on mancozeb; (b) RGR on azoxystrobin; (c) EC50 of mancozeb; and (d) EC50 of azoxystrobin

TABLE 2 Heritability and plasticity of mancozeb and azoxystrobin tolerance measured by the relative growth rate (RGR) in the six *Phytophthora infestans* populations collected from different host diversity

	Mancozeb			Azoxystrobin		
Diversity	Heritability	Plasticity	P:H	Heritability	Plasticity	P:H
1	0.054	0.063	1.16	0.052	0.087	1.65
2	0.036	0.061	1.72	0.010	0.089	8.82
3	0.032	0.076	2.37	0.019	0.116	6.22
4	0.047	0.056	1.19	0.019	0.148	7.99
5	0.022	0.075	3.46	0.035	0.104	2.95
6	0.024	0.053	2.24	0.023	0.148	6.55
Average	0.036	0.064	1.79	0.026	0.115	4.39
r	−0.748	−0.156	0.582	−0.250	0.745	0.163

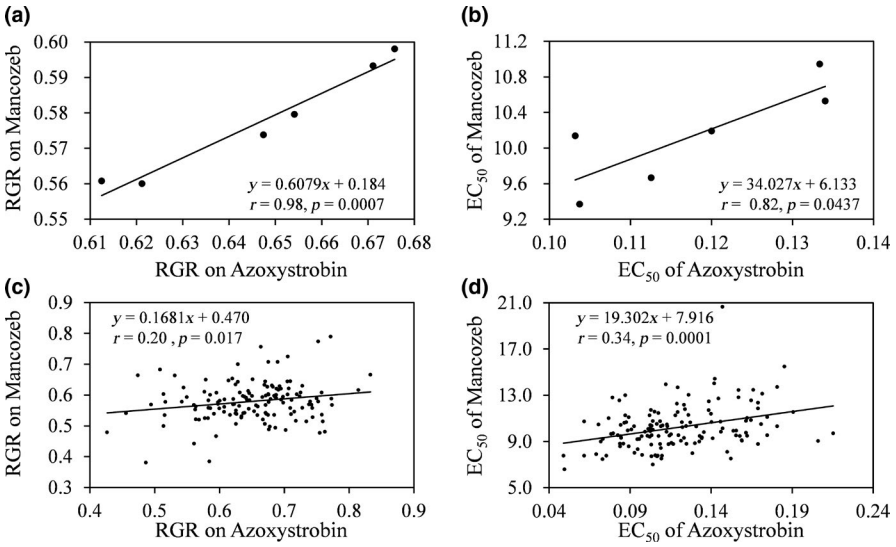


FIGURE 6 Associations between mancozeb and azoxystrobin tolerance in *Phytophthora infestans*: (a) population mean of relative growth rate (RGR); (b) population mean of EC50; (c) RGR of individual isolates; and (d) EC50 of individual isolates

epidemics, it is reasonable to believe that the pathogen isolates tested for fungicide tolerance originated from the same local source population consisted of many genotypes and hence had the same initial level of tolerance to the two fungicides. Although passage experiments documented that beneficial mutations might quickly emerge in *P. infestans* genomes after the pathogen was challenged by UV (Wang, 2021) and/or temperature stresses (Wu et al., 2020), we hypothesize that the unexpected results demonstrated in the

experiment were mainly caused by an adaptive process of *P. infestans* populations in response to selection imposed by host genetic diversity. This view is supported by the observed higher overall Q_{ST} than G_{ST} values in the comparative analysis of population differentiation between SSR markers and the measurements of fungicide tolerance, and by the positive association between population divergence in fungicide tolerance and host diversity level (Figure 3). Further analysis showed that host population diversity had a negative impact on *P. infestans* tolerance to mancozeb and azoxystrobin (Figure 4), suggesting that within-host population heterogeneity through cultivar mixing selects for pathogens with a higher fungicide sensitivity. We therefore argue that this host population diversity approach can be used along with other diversification strategies such as the spatial and temporal deployment of different chemical compounds (Lucas et al., 2015; Parnell et al., 2006), to sustainably maintain fungicide efficacy in agricultural ecosystems.

Variation is a key parameter determining the evolutionary adaptation of living organisms to environments. Pathogens with higher variation tend to response faster and adapt better to environmental stresses than those with lower variation (Zhan & McDonald, 2013; Zhan et al., 2015). One of the main concerns in adopting a heterogeneity approach to manage plant pathogens is that it may select for great pathogen variation, thereby increasing evolutionary potential of developing fungicide resistance (Lannou, 2001). The finding of a negative association between host potato diversity level and variation of *P. infestans* tolerance (Figure 5) indicates that host heterogeneity reduces the evolutionary potential and increases the time required to develop fungicide resistance in pathogens. In addition, previous results showed that host genetic diversification significantly reduced the aggressiveness and enhanced the latent period of pathogens, leading to significantly less severe disease outbreaks (Yang et al., 2019). Lower fungicide tolerance, mitigated aggressiveness and extended latent periods of pathogens associated with higher host populations diversity reduce the fungicide dose and application frequency needed to achieve the same level of disease control, relaxing selection pressure acting on pathogen populations (Burdon et al., 2016) thereby, further retarding the evolutionary development of fungicide resistance.

Both genetic differentiation (genetics) and plasticity (epigenetics) contribute to the evolutionary adaptation of biological traits to environments in pathogens (Huchard et al., 2014; Mohd-Assaad et al., 2016). However, the relative role of the two adaptive mechanisms can vary substantially among the traits. Genetic differentiation is determined by accumulative sequence variation in the genes associated with a phenotypic value of traits, while plasticity is a phenomenon whereby a genotype produces different phenotypes in response to environmental variation through changes in gene expression and/or enzymatic activity (Zhan & McDonald, 2011). Evolutionary adaptation resulting from both genetic and epigenetic phenomena is heritable (Pelletier et al., 2007) with genetic adaptation usually serving as a reinforcement of epigenetic adaptation (Draghi & Whitlock, 2012). We found that plasticity was several fold higher than the heritability irrespective of fungicide types and host diversity level (Table 2), indicating that plasticity plays a more important role in the development of fungicide resistance in *P. infestans*.

Mancozeb is a multi-target contact fungicide while azoxystrobin is a site-specific systemic fungicide. Resistance to site-non-specific fungicides is developed as a consequence of sequential gene mutations in many independent genes over the pathogen genome. Each mutation contributes quantitatively and accumulatively to the resistance (Lucas et al., 2015). On the other hand, resistance to site-specific fungicides usually emerges from single point mutations in target genes (Lucas et al., 2015). Interestingly, we found a positive association between the tolerances of *P. infestans* to mancozeb and azoxystrobin (Figure 6), indicating that the negative impact of host population genetic diversity on the evolution of fungicide resistance in *P. infestans* might be a general phenomenon and independent of biochemical features of fungicides (Figure 6). In addition to mutation of the target cytochrome b gene, resistance to azoxystrobin is an amalgam of multiple mechanisms involving an array of genetic, biochemical and/or physiological processes in pathogens. The altered expression of ATP-binding cassette transporters and major facilitator superfamily transporters (Matić et al., 2019; Stergiopoulos et al., 2002) are also deployed by plant pathogens to resist the impact of site-non-specific fungicides such as mancozeb (Cabrito et al., 2011). It is likely that reduced influxes or increased effluxes and detoxification of unrelated chemical compounds associated with synergistic changes in these genetic, biochemical and/or physiological processes by pleiotropy (Fernández-Ortuño et al., 2008; Omrane et al., 2015), rather than simultaneous mutations in the corresponding target genes, contribute to the enhanced efficacy of fungicides in controlling *P. infestans* under conditions of high host population genetic diversity. Pleiotropy together with the quantitative features generated by multiple resistance mechanisms may also contribute to the observed continuous variation of mancozeb and azoxystrobin tolerance in the pathogen populations (Figures 1 and 2; Figures S1 and S2).

Our findings have multifaceted implications not only for effective and durable fungicide management but also for sustainable development. One of the most pressing challenges in agriculture is how to ensure food security, resource conservation and socio-economic stability and development while maintaining healthy, sustainable ecosystems capable of quickly adapting to changing environments (Garibaldi et al., 2017; Tscharntke et al., 2012). Plant disease management in agriculture particularly involves many social, economic and ecological considerations that are not easily reconciled due to complex interactions that generate a range of trade-offs (Garibaldi et al., 2017; Tscharntke et al., 2012). Selection for increased fungicide efficacy (Figure 4), reduced evolutionary potential of pathogens (Yang et al., 2019; Zhu et al., 2000), decreased fungicide dose and application frequency, enhanced plant productivity, enriched soil microbial diversity (Yang et al., 2019) and increased nutrient efficiency through higher host population heterogeneity simultaneously improve the supply of high-quality food (less fungicide residue), farm income, resource conservation and ecological resilience, therefore, fulfil sustainable development goals (Toledo & Burlingame, 2006).

Although our results are derived from the potato–*P. infestans* interaction, similar contributions may also exist in other pesticides, antibiotic drugs or host–pathogen associations. This possibility is worthy of further pursuit.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 31901861 and 31460368).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHORS' CONTRIBUTIONS

Our study brings together authors from a number of different countries, including scientists in the country where the study was carried out. The authors are complementary in expertise in the relevant fields ranging from plant breeding, plant pathology, evolutionary biology, ecologists and field biologists, and all authors were engaged either with project initiation, experimental design, the generation, analysis interpretation of data and/or manuscript preparation to ensure that the diverse perspectives of the research are addressed. Whenever relevant, literature published by scientists from the region was cited. L.-N.Y. and O.N. collected and genotyped pathogen isolates, generated fungicide tolerance data, analysed data and wrote the manuscript; Z.-C.P. conducted field experiments, collected pathogen isolates and wrote the manuscript; Y.-P.W. and A.W. generated and analysed fungicide tolerance data; R.-S.C. and J.J.B. wrote the manuscript; Q.-J.S. conceived the project and managed the field experiment; J.Z. conceived, designed and supervised the experiments, analysed the data and wrote the manuscript. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.wh70rxwnq (Yang et al., 2021).

ORCID

Li-Na Yang https://orcid.org/0000-0003-4431-7213
Jiasui Zhan https://orcid.org/0000-0001-9250-0157

REFERENCES

Aguayo, J., Elegbede, F., Husson, C., Saintonge, F. X., & Marçais, B. (2014). Modeling climate impact on an emerging disease, the *Phytophthora alni*-induced alder decline. *Global Change Biology*, *20*, 3209–3221.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., & Parr-Dobrzanski, B. (2002). The strobilurin fungicides. *Pest Management Science*, *58*, 649–662. https://doi.org/10.1002/ps.520

Brunner, P. C., Stefansson, T. S., Fountaine, J., Richina, V., & McDonald, B. A. (2016). A global analysis of cyp51 diversity and azole sensitivity in *Rhynchosporium commune*. *Phytopathology*, *106*, 355–361.

Burdon, J. J., Barrett, L. G., Yang, L. N., He, D. C., & Zhan, J. (2020). Maximizing world food production through disease control. *BioScience*, *70*, 126–128. https://doi.org/10.1093/biosci/biz149

Burdon, J. J., Zhan, J., Barrett, L. G., Papaïx, J., & Thrall, P. H. (2016). Addressing the challenges of pathogen evolution on the world's arable crops. *Phytopathology*, *106*, 1117–1127. https://doi.org/10.1094/PHYTO-01-16-0036-FI

Cabrito, T. R., Teixeira, M. C., Singh, A., Prasad, R., & Sá-Correia, I. (2011). The yeast ABC transporter Pdr18 (ORF YNR070w) controls plasma membrane sterol composition, playing a role in multidrug resistance. *Biochemical Journal*, *440*, 195–202. https://doi.org/10.1042/BJ20110876

Creissen, H. E., Jorgensen, T. H., & Brown, J. K. (2016). Increased yield stability of field-grown winter barley (*Hordeum vulgare* L.) varietal mixtures through ecological processes. *Crop Protection*, *85*, 1–8. https://doi.org/10.1016/j.cropro.2016.03.001

Draghi, J. A., & Whitlock, M. C. (2012). Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation. *Evolution*, *66*, 2891–2902. https://doi.org/10.1111/j.1558-5646.2012.01649.x

Fernández-Ortuño, D., Torés, J. A., De Vicente, A., & Pérez-García, A. (2008). Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. *International Microbiology*, *11*, 1.

Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, *360*, 739–742. https://doi.org/10.1126/science.aap7999

Fontem, D. A., & Aighewi, B. (1993). Effect of fungicides on late blight control and yield loss of potato in the western highlands of Cameroon. *International Journal of Pest Management*, *39*, 152–155. https://doi.org/10.1080/09670879309371781

Fry, W. (2008). *Phytophthora infestans*: The plant (and R gene) destroyer. *Molecular Plant Pathology*, *9*, 385–402.

Garibaldi, L. A., Gemmill-Herren, B., D'Annolfo, R., Graeub, B. E., Cunningham, S. A., & Breeze, T. D. (2017). Farming approaches for greater biodiversity, livelihoods, and food security. *Trends in Ecology & Evolution*, *32*, 68–80. https://doi.org/10.1016/j.tree.2016.10.001

Grimmer, M. K., van den Bosch, F., Powers, S. J., & Paveley, N. D. (2015). Fungicide resistance risk assessment based on traits associated with the rate of pathogen evolution. *Pest Management Science*, *71*, 207–215. https://doi.org/10.1002/ps.3781

Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L., & Sheppard, B. (2010). Mancozeb: Past, present, and future. *Plant Disease*, *94*, 1076–1087. https://doi.org/10.1094/PDIS-94-9-1076

Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. *Journal of Chemical Biology*, *7*, 133–141. https://doi.org/10.1007/s12154-014-0113-1

Hawkins, N. J., & Fraaije, B. A. (2018). Fitness penalties in the evolution of fungicide resistance. *Annual Review of Phytopathology*, *56*, 339–360. https://doi.org/10.1146/annurev-phyto-080417-050012

He, M. H., Li, D. L., Zhu, W., Wu, E. J., Yang, L. N., Wang, Y. P., Waheed, A., & Zhan, J. (2018). Slow and temperature-mediated pathogen adaptation to a nonspecific fungicide in agricultural ecosystem. *Evolutionary Applications*, *11*, 182–192. https://doi.org/10.1111/eva.12526

Huchard, E., Charmantier, A., English, S., Bateman, A., Nielsen, J. F., & Clutton-Brock, T. (2014). Additive genetic variance and developmental plasticity in growth trajectories in a wild cooperative mammal. *Journal of Evolutionary Biology*, *27*, 1893–1904. https://doi.org/10.1111/jeb.12440

Jørgensen, L. N., Van den Bosch, F., Oliver, R. P., Heick, T. M., & Paveley, N. D. (2017). Targeting fungicide inputs according to need. *Annual Review of Phytopathology*, *55*, 181–203. https://doi.org/10.1146/annurev-phyto-080516-035357

Kirk, W. W., Felcher, K. J., Douches, D. S., Coombs, J., Stein, J. M., Baker, K. M., & Hammerschmidt, R. (2001). Effect of host plant resistance and reduced rates and frequencies of fungicide application to control potato late blight. *Plant Disease*, *85*, 1113–1118. https://doi.org/10.1094/PDIS.2001.85.10.1113

Kokalis-Burelle, N., Butler, D. M., & Rosskopf, E. N. (2013). Evaluation of cover crops with potential for use in anaerobic soil disinfestation (ASD) for susceptibility to three species of *Meloidogyne*. *Journal of Nematology*, *45*, 272.

Lamari, L. (2002). *Assess: Image analysis software for plant disease quantification*. APS Press.

Lannou, C. (2001). Intrapathotype diversity for aggressiveness and pathogen evolution in cultivar mixtures. *Phytopathology*, *91*, 500–510. https://doi.org/10.1094/PHYTO.2001.91.5.500

Lucas, J. A., Hawkins, N. J., & Fraaije, B. A. (2015). The evolution of fungicide resistance. *Advances in Applied Microbiology*, *90*, 29–92.

Lurwanu, Y., Wang, Y. P., Abdul, W., Zhan, J., & Yang, L. N. (2020). Temperature-mediated plasticity regulates the adaptation of *Phytophthora infestans* to azoxystrobin fungicide. *Sustainability*, *12*, 1188. https://doi.org/10.3390/su12031188

Matić, S., Gilardi, G., Gisi, U., Gullino, M. L., & Garibaldi, A. (2019). Differentiation of *Pythium* spp. from vegetable crops with molecular markers and sensitivity to azoxystrobin and mefenoxam. *Pest Management Science*, *75*, 356–365.

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59*, 70–80. https://doi.org/10.1111/j.0014-3820.2005.tb00895.x

Mohd-Assaad, N., McDonald, B. A., & Croll, D. (2016). Multilocus resistance evolution to azole fungicides in fungal plant pathogen populations. *Molecular Ecology*, *25*, 6124–6142. https://doi.org/10.1111/mec.13916

Nei, M. (1987). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*, 583–590. https://doi.org/10.1093/genetics/89.3.583

Omrane, S., Sghyer, H., Audéon, C., Lanen, C., Duplaix, C., Walker, A. S., & Fillinger, S. (2015). Fungicide efflux and the MgMFS 1 transporter contribute to the multidrug resistance phenotype in *Zymoseptoria tritici* field isolates. *Environmental Microbiology*, *17*, 2805–2823.

Ott, R. L. (1992). *An introduction to statistical methods and data analysis* (5th ed.). Duxbury Press.

Parnell, S., Van Den Bosch, F., & Gilligan, C. A. (2006). Large-scale fungicide spray heterogeneity and the regional spread of resistant pathogen strains. *Phytopathology*, *96*, 549–555. https://doi.org/10.1094/PHYTO-96-0549

Pelletier, F., Reale, D., Garant, D., Coltman, D. W., & Festa-Bianchet, M. (2007). Selection on heritable seasonal phenotypic plasticity of body mass. *Evolution*, *61*, 1969–1979. https://doi.org/10.1111/j.1558-5646.2007.00160.x

Rex Consortium. (2013). Heterogeneity of selection and the evolution of resistance. *Trends in Ecology & Evolution*, *28*, 110–118.

Stecher, B., Maier, L., & Hardt, W. D. (2013). 'Blooming' in the gut: How dysbiosis might contribute to pathogen evolution. *Nature Reviews Microbiology*, *11*, 277–284.

Stergiopoulos, I., Zwiers, L. H., & De Waard, M. A. (2002). Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporters of the ATP-binding cassette and major facilitator superfamily. *European Journal of Plant Pathology*, *108*, 719–734.

Toledo, Á., & Burlingame, B. (2006). Biodiversity and nutrition: A common path toward global food security and sustainable development. *Journal of Food Composition and Analysis*, *19*, 477–483. https://doi.org/10.1016/j.jfca.2006.05.001

Tonsor, S. J., Elnaccash, T. W., & Scheiner, S. M. (2013). Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. *Evolution*, *67*, 2923–2935. https://doi.org/10.1111/evo.12175

Tscharntke, T., Clough, Y., Wanger, T. C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J., & Whitbread, A. (2012). Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, *151*, 53–59. https://doi.org/10.1016/j.biocon.2012.01.068

van den Bosch, F., Oliver, R., Berg, F. V. D., & Paveley, N. (2014). Governing principles can guide fungicide-resistance management tactics. *Annual Review of Phytopathology*, *52*, 175–195. https://doi.org/10.1146/annurev-phyto-102313-050158

Wang, Y. P. (2021). *The biology and mechanisms of UV adaptation in the pathogen* Phytophthora infestans *causing potato late blight* (PhD dissertation). Fujian Agriculture and Forestry University, Fuzhou, China.

Wu, E. J., Wang, Y. P., Yahuza, L., He, M. H., Sun, D. L., Huang, Y. M., Liu, Y. C., Yang, L. N., Zhu, W., & Zhan, J. (2020). Rapid adaptation of the Irish potato famine pathogen *Phytophthora infestans* to changing temperature. *Evolutionary Applications*, *13*, 768–780.

Wu, E. J., Yang, L. N., Zhu, W., Chen, X. M., Shang, L. P., & Zhan, J. (2016). Diverse mechanisms shape the evolution of virulence factors in the potato late blight pathogen *Phytophthora infestans* sampled from China. *Scientific Reports*, *6*, 26182. https://doi.org/10.1038/srep26182

Yang, L. -N., Nkurikiyimfura, O., Pan, Z. -C., Wang, Y. -P., Waheed, A., Chen, R. -S., Burdon, J. J., Sui, Q. -J., & Zhan, J. (2021). Data from: Plant diversity ameliorates the evolutionary development of fungicide resistance in an agricultural ecosystem. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.wh70rxwnq

Yang, L. N., Pan, Z. C., Zhu, W., Wu, E. J., He, D. C., Yuan, X., Qin, Y. Y., Wang, Y., Chen, R. S., Thrall, P. H., Burdon, J. J., Shang, L. P., Sui, Q. J., & Zhan, J. (2019). Enhanced agricultural sustainability through within-species diversification. *Nature Sustainability*, *2*, 46–52. https://doi.org/10.1038/s41893-018-0201-2

Yang, L. N., Zhu, W., Wu, E. J., Yang, C., Thrall, P. H., Burdon, J. J., Jin, L. P., Shang, L. P., & Zhan, J. (2016). Trade-offs and evolution of thermal adaptation in the Irish potato famine pathogen *Phytophthora infestans*. *Molecular Ecology*, *25*, 4047–4058.

Yang, R. C., Yeh, F. C., & Yanchuk, A. D. (1996). A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp. *latifolia* by FST. *Genetics*, *142*, 1045–1052.

Zhan, J., & McDonald, B. A. (2011). Thermal adaptation in the fungal pathogen *Mycosphaerella graminicola*. *Molecular Ecology*, *20*, 1689–1701. https://doi.org/10.1111/j.1365-294X.2011.05023.x

Zhan, J., & McDonald, B. A. (2013). Experimental measures of pathogen competition and relative fitness. *Annual Review of Phytopathology*, *51*, 131–153. https://doi.org/10.1146/annurev-phyto-082712-102302

Zhan, J., Mundt, C. C., Hoffer, M. E., & McDonald, B. A. (2002). Local adaptation and effect of host genotype on the rate of pathogen evolution: An experimental test in a plant pathosystem. *Journal of Evolutionary Biology*, *15*, 634–647. https://doi.org/10.1046/j.1420-9101.2002.00428.x

Zhan, J., Mundt, C. C., & McDonald, B. A. (2001). Using restriction fragment length polymorphisms to assess temporal variation and estimate the number of ascospores that initiate epidemics in field populations of *Mycosphaerella graminicola*. *Phytopathology*, *91*, 1011–1017.

Zhan, J., Stefanato, F. L., & McDonald, B. A. (2006). Selection for increased cyproconazole tolerance in *Mycosphaerella graminicola* through local adaptation and in response to host resistance. *Molecular Plant Pathology*, *7*, 259–268.

Zhan, J., Thrall, P. H., & Burdon, J. J. (2014). Achieving sustainable plant disease management through evolutionary principles. *Trends in Plant Science*, *19*, 570–575. https://doi.org/10.1016/j.tplants.2014.04.010

Zhan, J., Thrall, P. H., Papaïx, J., Xie, L., & Burdon, J. J. (2015). Playing on a pathogen's weakness: Using evolution to guide sustainable plant disease control strategies. *Annual Review of Phytopathology*, *53*, 19–43. https://doi.org/10.1146/annurev-phyto-080614-120040

Zhu, W., Yang, L. N., Wu, E. J., Qin, C. F., Shang, L. P., Wang, Z. H., & Zhan, J. (2015). Limited sexual reproduction and quick turnover in the population genetic structure of *Phytophthora infestans* in Fujian, China. *Scientific Report*, *5*, 10094. https://doi.org/10.1038/srep10094

Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J. X., Yang, S., Hu, L., Leung, H., Mew, T. W., Teng, P. S., Wang, Z., & Mundt, C. C. (2000). Genetic diversity and disease control in rice. *Nature*, *406*, 718–722. https://doi.org/10.1038/35021046

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Yang, L.-N., Nkurikiyimfura, O., Pan, Z.-C., Wang, Y.-P., Waheed, A., Chen, R.-S., Burdon, J. J., Sui, Q.-J., & Zhan, J. (2021). Plant diversity ameliorates the evolutionary development of fungicide resistance in an agricultural ecosystem. *Journal of Applied Ecology*, 58, 2566–2578. https://doi.org/10.1111/1365-2664.13978