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ScienceDirect
Journal of Theoretical Biology 252 (2008) 621–632
Journal of Theoretical Biology
www.elsevier.com/locate/yjtbi

Adaptation to the cost of resistance in a haploid clonally reproducing organism: The role of mutation, migration and selection

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Received 13 June 2007; received in revised form 25 January 2008; accepted 16 February 2008
Available online 26 February 2008

Abstract

A model of compensatory evolution with respect to fungicide resistance in a haploid clonally reproducing fungus is developed in which compensatory mutations mitigate fitness costs associated with resistance. The role of mutation, migration and selection in invasion of rare genotypes when the environment changes from unsprayed to sprayed and from sprayed to unsprayed is analysed in detail. In some circumstances (ignoring back mutations) stable internal steady-state values for multiple genotypes can be obtained. In these cases a threshold value (f^*) for the fraction of the population exposed to the fungicide can be derived for the transition between different steady-state conditions. Conditions are derived for invasion-when-rare of resistant genotypes at boundary equilibria established sometime after the onset of spraying and conversely of sensitive genotypes sometime after the cessation of spraying are derived. In these cases conditions are presented for (a) the invasion of a resistant genotype with a compensatory mutation (resistant-compensated) into a sensitive-uncompensated population that has re-equilibrated following the onset of spraying and (b) the invasion of a susceptible-uncompensated genotype into a resistant-compensated population that has re-equilibrated following the cessation of spraying, provided certain conditions are met. A resistant-compensated genotype may be fixed (or at near-fixation) in the population following a period of spraying, provided the mean intrinsic growth rate of the resistant-compensated genotype in a sprayed environment (over exposed and non-exposed parts of the population) is greater than that of the susceptible-uncompensated genotype. The fraction of the population exposed (the efficiency of spraying) is critical in this respect. However, it is possible for a sensitive-uncompensated genotype to invade provided there is no fitness gain associated with the resistant-compensated genotype, introduction by migration occurs following equilibration of the population to the new environment, and competitive effects are re-imposed when spraying ceases. We further derive a threshold level for the resident resistant-compensated population to reduce to following the cessation of spraying, such that the introduced susceptible-uncompensated genotype will invade. These results will be of use in determining the long-term persistence of resistance in a pathogen population once a fungicide is no longer effective and removed from use.

Keywords: Fungicide resistance; Compensatory evolution; Mathematical model; Selection–mutation balance; Invasion-when-rare

1. Introduction

Resistance to chemicals used to control populations of pathogenic microorganisms often develops on rapid time scales (Palumbi, 2001), a major concern for agricultural systems, where pesticides are commonly used. For pesticides, there have been international efforts to develop recommendations for rational resistance management, but despite these efforts resistance to newly introduced fungicides has continued to pose significant problems (Gullino et al., 2000). Mathematical models for the rate of selection for fungicide resistance have been proposed (Skylakakis, 1982; Shaw, 1989), other models have been applied to the question of how best to reduce the rate at which resistance develops in a population (Birch and Shaw, 1997; Hall et al., 2004) and how this depends on spray heterogeneity and the spatial scale considered (Shaw, 2000; Parnell et al., 2005, 2006).

A key question in resistance studies is the extent to which resistant strains decline when fungicide selection is removed, and whether they persist in the population.

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0022-5193/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtbi.2008.02.023

A decline is expected when resistant strains have lower fitness than sensitive strains in the absence of fungicide, unless there are compensatory mechanisms which restore or even increase fitness, the main focus of this paper. The literature on persistence of fungicide resistance is limited and probably biased so it is difficult to generalise. In some cases, such as that reported for quinoxyfen-resistant barley powdery mildew (*Erysiphe graminis* f. sp. *hordei*), laboratory and field isolates were found to be defective for sporulation and required the presence of quinoxyfen for survival in culture (Hollomon et al., 1997). In other cases dicarboximide-resistant isolates of *Botrytis cinerea* survived less well than sensitive isolates as sclerotia, but not as mycelia (Raposo et al., 2000). Sensitivity to sterol-demethylation-inhibiting (DMI) fungicides in *Cercospora beticola* decreased with prolonged DMI use, however less-sensitive strains declined in frequency during the over-wintering period due either to a reduced saprophytic ability or a lower competitive ability in the absence of DMI exposure (Karaoglanidis et al., 2002). A lower competitive ability was observed in overwintered isolates of *E. graminis* f. sp. *tritici* with reduced sensitivity to fenpropimorph compared with natural migrants into the experimental field (Engels and deWaard, 1996).

Resistance to fungicides has also been reported to persist rather than decline in populations when selection is removed, although there has been no comprehensive study on this as far as we are aware. Examples include carbendazim- (but not dicarboximide-) resistance in *Monilinia fructicola* (Sanoamuang and Gaunt, 1995) and insensitivity to mefenoxam in *Phytophthora capsici* (Lamour and Hausbeck, 2001). There may be serious implications for the persistence of resistance in the absence of selection. For example, fungicide resistance in *Fusarium culmorum* was reported to be accompanied by a more persistent mycotoxin production (D'Mello et al., 1998). In *Venturia inaequalis*, resistance to DMI fungicides appears to a greater extent in phenotypes resistant to previously used, unrelated fungicides such as the benzimidazoles (Koller and Wilcox, 2001).

The issue of whether resistance persists is intrinsically linked to whether there are fitness costs associated with resistance. On this the literature is again equivocal. Resistance to DMI fungicide was identified in *Sclerotinia homoeocarpa* (Hsiang et al., 1998), in areas where the fungicide had not been used. Results in field trials on unsprayed host plants indicated resistance-related fitness costs in less-sensitive isolates. In other cases fitness costs were not identified consistently in field isolates of *Phytophthora infestans* resistant to metalaxyl (Matuszak et al., 1994). However, the pattern of persistence observed when a selective agent is removed is difficult to interpret solely in terms of costs of fitness associated with antibiotic and pesticide resistance (Peck, 2001; Wijngaarden et al., 2005). Second-site mutations, occurring at different loci from the resistance mutation, can play a major role in compensating for a cost of resistance. It is important to note that such compensatory mutations can be independently deleterious in the absence of the resistance mutation (Maisnier-Patin and Andersson, 2004). The compensatory mutations may then enable resistance mutations to remain in the population when chemical control ceases. Clearly, there is a need for understanding the conditions that favour compensatory mutations. Here we focus on fungicide resistance because of: (i) the major impact on disease management in many economic crop plants and (ii) models of resistance development in the plant pathology literature have not previously considered the effects of compensatory evolution (Parnell et al., 2006), other than the population genetics model described previously by the authors (Wijngaarden et al., 2005).

In this paper, we modify our earlier model through an epidemiological approach dealing explicitly with both resistance and compensatory mutations, although the main emphasis is on the fate of rare migrant genotypes when introduced into a resident population. We are aware of only one epidemiological model of resistance development that includes compensatory mutations. Handel et al. (2006) developed a model for gonorrhea dynamics combining between-host dynamics and within-host conversion rates from sensitive to resistant-uncompensated to resistant-compensated strains. The results of our study may also have broad applicability for understanding the evolution of adaptation in populations of predominantly haploid, clonally reproducing organisms. In such populations, changes in the numbers of the different genotypes result from differential growth rates among clones so we will study their dynamics by linked differential equations describing density-dependent population growth, migration and mutation. A potential problem in this approach through differential growth rates is the multidimensionality that can result. In considering compensatory evolution we develop a model for four sub-populations in two environments (exposed and non-exposed). In principle, this could involve eight differential growth rates, one for each sub-population $\times$ environment combination. We avoid this problem by defining two basic growth rates for the two environments, and then modify these by fitness costs associated with resistance and compensatory alleles. Previous epidemiological models of resistance development have taken different approaches to incorporating fitness costs (see Hall et al., 2004): these are either not included, represented through selection coefficients, or subsumed in the growth rates (Skylakakis, 1982) and/or basic reproductive number (Gubbins and Gilligan, 1999). By modifying the growth rates in this way we aim at describing both the epidemiological and evolutionary dynamics of adaptation.

2. The model

The motivation for this study is to identify circumstances in which resistant genotypes remain in a pathogen population once selection (i.e. exposure to a fungicide)