

1 **Increasing ecological heterogeneity can constrain biopesticide resistance evolution**

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28 **Keywords:**

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30 Sustainable agriculture; agroecology; crop protection; integrated pest management; landscape
31 diversity; genotype-by-environment interactions.

32

33

34 **Abstract**

35

36 Microbial biopesticides containing living parasites are valuable emerging crop protection
37 technologies against insect pests, but they are vulnerable to resistance evolution.

38 Fortunately, the fitness of alleles that provide resistance, including to parasites used in
39 biopesticides, frequently depends on parasite identity and environmental conditions. This
40 context-specificity suggests a sustainable approach to biopesticide resistance management
41 through landscape diversification. To mitigate resistance risks, in addition to increasing the
42 range of biopesticides available to farmers, we advocate simultaneously encouraging other
43 aspects of landscape-wide crop heterogeneity that can generate variable selection on
44 resistance alleles. This approach requires agricultural stakeholders to prioritise diversity as
45 well as efficiency, both within agricultural landscapes and the biocontrol marketplace.

46

47 ***Biopesticides are important products for ecologically sustainable crop protection***

48

49 The prevalence and adaptive capacity of insect pests cause huge problems for food security.
50 Insects consume as much as 20% of crops while growing or in storage [1], which represents a
51 large fraction of the future improvements needed to feed the growing human population [2].
52 Despite considerable research, pest control methods continue to suffer reduced effectiveness
53 due to **pesticide resistance** (See Glossary), leading to crop failures, economic losses, and
54 food insecurity [2–4]. New pest control technologies, including **microbial biopesticides** and
55 other **biological control** agents, provide a welcome addition to the arsenal of crop protection
56 methods [5]. These biological agents are vital tools in **integrated pest management** (IPM)
57 and can be used instead of synthetic products that have suffered **resistance evolution** or
58 legislative restriction. Biocontrol provides attractive crop protection options due to minimal
59 adverse effects on human health, promotion of ecosystem services, and compatibility with
60 organic farming requirements [6]. Globally, biopesticide use is increasing by almost 10% per
61 year [7]. Although the worldwide microbial biopesticide market was previously dominated
62 almost exclusively by products based on *Bacillus thuringiensis* (Bt) (95% in the 1990s), the
63 range of microbial bioinsecticides has increased substantially [8]. In this article we focus on
64 microbial biopesticides formulated from the living pathogens of insect crop pests. We argue
65 that resistance management approaches need to be implemented for these crop protection
66 products and propose new solutions. We place our argument into the context of other well-
67 developed resistance management frameworks that are already implemented for synthetic
68 insecticides and **transgenic crops** (such as those incorporating Bt pesticidal molecules).

69

70 ***Biopesticides present new opportunities for resistance management***

71

72 To deliver ecologically sustainable crop protection the transition from synthetic pesticides to
73 microbial biopesticides and other forms of biological control must accelerate; this will require
74 new biological agents to come to market accompanied by increased adoption by farmers.
75 However, expansions in microbial biopesticide usage will increase selection pressures on
76 pests to develop resistance and therefore justifies careful consideration of approaches to
77 proactively mitigate the risks of resistance evolution [9]. Although resistance management for
78 transgenic crops is well developed [10,11], until now resistance management for living
79 biological control agents has been relatively neglected.

80

81 Notwithstanding early assertions that they would not incite resistance evolution [12], there is
82 already considerable evidence that resistance to microbial biopesticides can evolve in the
83 field and also in lab studies (Box 1). Following deployment of granulovirus based
84 insecticides for control of codling moth (*Cydia pomonella*) in European apple orchards in the
85 early 1990s, resistance developed by the mid-2000s requiring development of novel products
86 with different viral strains [13]. Perhaps the most famous viral biocontrol agent of all time,
87 *Myxoma* virus, rapidly triggered resistance evolution in rabbits (*Oryctolagus cuniculus*) by
88 selecting on pre-existing variation in immune system genes [14]. Similarly, some major pest
89 species have developed resistance to the most widely used bacterial biopesticide in the world:
90 *B. thuringiensis* [15]. Alarming, there is even recent evidence of substantially elevated
91 resistance to classical biological control **parasitoids** under field settings [16]. With this
92 increasing evidence base, microbial biopesticides must be protected to avoid them suffering
93 the same resistance fate as chemical pesticides.

94

95 ***How is resistance to microbial biopesticides different to other crop protection products?***

96

97 In the case of synthetic pesticides and transgenic crops, resistance alleles frequently have
98 binary effects on phenotype conferring orders of magnitude decrease in susceptibility to the
99 agent. This is in part due to the relatively simple nature of molecular interactions between
100 these control products and the pest molecules they target, which enables resistant phenotypes
101 to arise from genetic changes at single or a small number of loci [17,18]. [19,20]. In contrast,
102 resistance to living microbial biocontrol agents should involve more genes because living
103 organisms are by necessity more complex than individual biomolecular compounds. It is
104 worth noting for example that while resistance to living *B. thuringiensis* is rare, resistance to
105 the specific insecticidal proteins produced by transgenic crops occurs more frequently [21].
106 Resistance to living organisms such as those in fungal biopesticides will therefore often be
107 determined by multiple gene loci, where individual alleles may have only small effects on
108 susceptibility [22]; as a consequence, susceptibility to such biopesticides typically varies
109 continuously among individuals [23].

110

111 However, drawing general conclusions about resistance to all biopesticides is difficult
112 because biopesticides and other biocontrol agents encompass a wide spectrum of natural
113 enemies for which the evolutionary basis of pest resistance differs considerably. In Box 1 we
114 place microbial biopesticides containing living agents in the context of a continuum of crop

115 protection approaches for which the complexity of the genomic architecture of resistance
116 varies from relatively simple (e.g., chemical insecticides and some transgenic crop varieties)
117 through to complex (e.g., insects deployed in classical biological control). This variation in
118 genetic complexity has profound consequences for resistance evolution.

119

120 *Established resistance management strategies*

121

122 The evolutionary genetic assumptions underlying classical resistance management theory are
123 that resistance is usually genetically simple and underpinned by one or a few loci; alleles
124 conferring resistance are rare (and therefore predominantly present in heterozygotes); and
125 resistance alleles confer fitness costs in the absence of the pesticide, creating trade-offs to
126 pesticide resistance [24]. Crucially, while these assumptions are generally supported for
127 synthetic pesticides [25], they probably do not hold for some classes of biopesticides (Box 1),
128 which necessitates a different approach to microbial biopesticide resistance management.
129 Whilst the complexity of the genetic interactions between biopesticides and their hosts may
130 reduce the risks of resistance evolution, we do not think that this complexity on its own is
131 sufficient to prevent resistance evolution in many ecologically homogeneous agricultural
132 landscapes.

133

134 Resistance management targeted toward synthetic insecticides and transgenic crops has a
135 long pedigree in research and agricultural application. These resistance management
136 strategies can be placed into three broad groups.

137 First are strategies that seek to limit the opportunities for resistance alleles to spread in spite
138 of selection for resistance: in GM-crop systems, crop **refuges** encourage resistant individuals
139 to mate with susceptible individuals to generate susceptible offspring; whereas pyramid-Bt
140 varieties express multiple toxins with the aim that single step mutations will not confer
141 resistance.

142 Second, many approaches aim to reduce the long-term intensity of selection for pesticide
143 resistance, for example by minimising pesticide use through the adoption of IPM alternatives,
144 or through temporal **pesticide rotations** where a single active ingredient is only used
145 intermittently. These strategies rely on the principle that resistance alleles only confer high
146 fitness in the presence of one chemical agent; then in the absence of that agent, costs of
147 resistance cause allele frequencies to gradually decline. By cycling through pesticides with
148 distinct **modes of action** farmers could keep resistance at a low level. Unfortunately, costs of

149 resistance can be inconsistent across habitats [25], which can hamper their ability to constrain
150 resistance evolution.

151 Third, a conceptually more attractive modification of standard pesticide rotations involves
152 alternating between groups of pesticides that exhibit “negatively correlated cross-resistance”
153 (hereafter NCC-R), in which alleles conferring resistance to one pesticide directly impair the
154 ability to resist another, resulting in strong trade-offs [26]. This approach differs
155 fundamentally from a pesticide rotation because the management strategy is designed to drive
156 down the frequency of resistance alleles (using an alternative pesticide) rather than simply
157 relying on the general fitness costs of resistance to erode previous partial selective sweeps of
158 resistance. Despite this theoretical promise, the ability of these trade-offs to prevent
159 resistance evolution has not often been realised [27]: even if two pesticides confer NCC-R,
160 the genetic associations that produce trade-offs can themselves evolve over time, and lead to
161 positive cross-resistance (in which insects resistant to one pesticide are also resistant to
162 others) [28]. When these genetic associations involve a small number of loci, recombination
163 to produce positive cross-resistance can happen relatively easily, meaning the efficacy of
164 NCC-R in managing resistance can be short lived [29]. However, the promise of NCC-R for
165 generating variable selection is much greater for control methods for which resistance is
166 under complex polygenic genetic control, such as for biopesticides containing living
167 organisms.

168

169 Until now, strategies to manage pesticide resistance through heterogeneous selection
170 pressures have principally sought to achieve it by creating diversity in the selective agents
171 themselves, and in their presence or absence. Here, we suggest that inconsistent selection for
172 resistance to a (potentially) single agent can be delivered by diversifying other aspects of the
173 agricultural environment.

174

175 *Why the evolutionary ecology of pathogens is particularly prone to inconsistent selection*

176

177 Strong selection pressures do not always drive rapid evolutionary change [30,31]. Natural
178 host-parasite systems illustrate how variable selection can sustain genetic variation for
179 infection susceptibility despite strong selection. Even though successful parasite defence
180 must provide a major fitness advantage, host populations almost ubiquitously exhibit high
181 genetic variability for parasite resistance traits [32]. In some host-parasite systems
182 coevolutionary interactions prevent resistance allele fixation through **Red Queen Dynamics**

183 [33,34] (Note that in contrast to natural systems, coevolution between biopesticides or
184 inundatively released biocontrol is impossible because the control agent is grown from stock
185 in the lab, rather than cultivated). However, more generally, selection in the tangled bank of
186 ecological systems is inconsistent due to environmental variation [35]. Parasites can exert
187 strong selection on hosts without driving fixation of resistance alleles because parasites are
188 usually genetically diverse, host-parasite interactions are often mechanistically complex, and
189 the outcomes of these interactions are frequently context-dependent. This context dependence
190 has been quantified in the form of “**genotype-by-environment**” **interactions** (GEIs), in
191 which the fitness of resistance alleles depends on the specific environment an organism
192 inhabits (Box 2).

193
194 Unfortunately, most modern agricultural cropping systems are highly homogenous, which
195 means that selection does not vary dramatically at a landscape scale (whole farms, and indeed
196 farming regions, frequently specialise on growing a narrow range of crop plants). Yet, natural
197 systems are far more diverse, meaning that multiple aspects of the environment vary
198 continuously, including the ambient conditions, the nature and quality of food, the presence
199 of symbiont, and the genotypes of competitors, pathogens and predators. It is this diversity
200 that favours unique multilocus genotypes at many different loci depending on the precise
201 ecological context. Can modern agricultural landscapes be engineered to similarly benefit
202 from the power of GEIs, to sustain genetic diversity and prevent resistance evolution to
203 biopesticides?

204 205 *Successfully exploiting GEIs for pest resistance management*

206
207 Our vision is to exploit GEIs to make biopesticide-based pest control more ecologically and
208 evolutionarily sustainable. The orthodox framework for pesticide resistance management
209 focusses on trying to delay evolution. While this approach limits pest adaptation, we instead
210 advocate harnessing the evolution of pests using the variable selection pressures generated by
211 heterogeneous landscapes. By keeping aspects of the pest landscape in sufficient flux,
212 selection for resistance will not be directional at a landscape scale: as the agricultural habitat
213 changes, the alleles favoured by selection will also change. This way local selection in any
214 one generation will result in evolution that takes the population away from the optimum
215 genotype to survive pest control measures in other distinct patches (or times) within the
216 heterogeneous landscape. Importantly, our approach need not require sacrificing some of the

217 crop as refuge, or foregoing pest control altogether, provided that the conditions under which
218 control occurs are sufficiently diverse to prevent directional selection on a landscape scale.

219

220 The heterogeneity we call for to manage biopesticide resistance will require altered farm
221 management at a landscape scale, but need not be substantially at odds with agricultural
222 productivity. Both **temporal rotations** and **spatial rotations** of heterogenous landscape
223 patches could be used by farmers to generate the required inconsistent selection (Box 3). The
224 greater the difference between two habitats in which a pest lives, the more likely it is that
225 multi-locus genotypes that promote performance in one habitat negatively affect performance
226 in the second. Our approach will be maximally effective if habitat patches differ in as many
227 ecological dimensions as possible. However, we recognise the tension between maximising
228 heterogeneity and maximising farm efficiency.

229

230 An obvious way of generating heterogeneity is to alternate the species or strain of pathogen
231 used in biopesticide products (as in chemical insecticide rotations, and consistent with IPM).
232 Trade-offs for resistance are nearly ubiquitous in host-pathogen interactions [36] and derive
233 mainly from two sources. First, strong resistance specificity means that combatting one
234 pathogen can make an organism more susceptible to others (mirroring concepts of NCC-R).
235 Second, investment in resistance may deprive organisms of the ability to invest in other life
236 history traits like reproduction and growth. Strong GEI for pathogen resistance is much more
237 likely than for chemical insecticides due to this specificity and the typically polygenic genetic
238 basis of resistance to natural enemies [37,38]. The biopesticide market is currently dominated
239 by products containing a relatively narrow diversity of pathogen strains [39]. Unlike synthetic
240 insecticides, where the development of new products with novel modes of action is usually
241 slow, the biological world provides us with an almost limitless array of natural pathogen
242 strains which could be harnessed as biopesticides.

243

244 It is our opinion that microbial biopesticides offer a highly novel way to generate inconsistent
245 selection for resistance. Farmers could alter other landscape dimensions (in addition to
246 altering pest control methods) for which pest fitness traits are likely to be underpinned by
247 complex multi-locus genotypes. Potential examples include the microbial community
248 associated with crop plants, or the pest diet (determined by crop varieties or crop species in
249 the case of **polyphagous pests**). Such environmental contexts are well known to change
250 selection on resistance genes: costs of resistance to *B. thuringiensis* are environmentally

251 dependent and vary depending on crop plant type [40], furthermore, exposure to additional
252 pathogens may help sustain genetic variation in resistance to *Bt* insecticidal proteins [41].

253

254 The options for crop diversification to generate GEIs for biopesticide resistance may be
255 greatest for polyphagous pests, not least because their interactions with different host plants
256 are likely to involve many genes. Polyphagous pests are among the most notorious species
257 for resistance evolution to chemical insecticides [42], perhaps due to prolonged coevolution
258 with the diverse secondary compounds plants have evolved for their own defence [43].

259 Because polyphagous insects so readily evolve resistance to synthetic insecticides and
260 transgenic crop varieties (e.g., see Table 1), biopesticides are particularly valuable control
261 agents for these species. We see clear opportunities for generating fluctuating selection on
262 these pests through GEIs if farmers diversify the crop species cultivated in the agricultural
263 landscape (e.g., see Box 3).

264

265 Many insect pests are active dispersers generating considerable **gene flow** among
266 populations. Therefore, the heterogeneity we endorse need only be coarse-grained across the
267 farming ecosystem. Whilst the precise details will vary between pests, heterogeneity at the
268 between-field or between-farm scale would probably be sufficient to forestall resistance
269 evolution for most pests. Although there may be additional benefits to finer-scale
270 heterogeneity (such as field margins, refuges or intercropping, which provide well-
271 demonstrated ecological benefits [44,45]) these are unlikely to be necessary to manage
272 resistance. Our approach of managing resistance evolution risks through crop heterogeneity
273 may therefore mean that resistance management strategies could deliver the parallel
274 ecological benefit of enhancing agricultural biodiversity to maximise ecosystem service
275 delivery, further incentivising the diversification of agricultural landscapes [46,47].

276

277

278 Table 1: Major agricultural arthropod pests that are polyphagous and the number of host plant
 279 genera they feed on [48]. Polyphagous species may be particularly well suited for biopesticide
 280 resistance management by manipulating crop plant diversity in the landscape.
 281

Order	Pest species	Common name	No of host plant genera	No. of pesticide active ingredients reported resistant
Insects				
Diptera	<i>Drosophila suzukii</i>	Spotted wing drosophila	63	1
Hemiptera	<i>Bemisia tabaci</i>	Silverleaf whitefly	36	56
	<i>Myzus persicae</i>	Green peach aphid	95	80
Lepidoptera	<i>Chrysodeixis includens</i>	Soybean looper	82	2
	<i>Cydia pomonella</i>	Codling moth	6	22
	<i>Helicoverpa armigera</i>	Cotton bollworm	50	49
	<i>Helicoverpa zea</i>	Corn earworm	58	21
	<i>Plutella xylostella</i>	Diamondback moth	15	95
	<i>Spodoptera frugiperda</i>	Fall armyworm	72	39
Thysanoptera	<i>Tuta absoluta</i>	Tomato leaf miner	11	14
	<i>Frankliniella occidentalis</i>	Western flower thrips	64	30
Arachnids				
Trombidiformes	<i>Tetranychus urticae</i>	Red spider mite	80	95

282

283 **Concluding remarks**

284

285 Pesticide resistant insects are among the most important and expensive obstacles to food
 286 security. Conventional chemical pesticides will continue to face heightened regulation and
 287 scrutiny, resulting in fewer products on the market, and creating more opportunities for new
 288 biopesticides. It would be a mistake to continue to intensively overuse individual microbial
 289 biopesticide products, and thereby hasten resistance evolution. Instead, we must protect these
 290 emerging pest control products to avoid the same problems of resistance as chemical

291 pesticides. From the industry perspective, it would be beneficial to create incentives for the
292 development of novel products in parallel (rather than launching new products only once
293 legislation or resistance has rendered previous products obsolete), and to alter licensing
294 frameworks to make registration of new biopesticides more straightforward. Such actions will
295 require care, especially in light of the highly variable and uncertain global pesticides market
296 [49]. Our proposal requires further research (see **Outstanding Questions**) and also presents
297 some challenges in adjusting prevailing attitudes on the importance of diversity in the market
298 and landscape. However, the promise of our approach justifies further effort: a landscape that
299 does not sacrifice livelihoods, environment, or food quality, but that in its embrace of
300 diversity makes for more resilient and evolutionarily sustainable food production.

301

302 *Acknowledgments*

303

304 All authors were supported by a joint Newton Fund international partnership between the
305 Biotechnology and Biological Sciences Research Council (BBSRC) in the UK and the São
306 Paulo Research Foundation (FAPESP) in Brazil under BBSRC award reference
307 BB/S018956/1 and FAPESP award reference 2018/21089-3. Additionally, LFB was
308 supported by a grant from Vetenskapsrådet (Sweden): 2021-05466, and MT by BBSRC
309 Pathfinder Grant BB/T010142/1. We are grateful to three anonymous reviewers for helpful
310 suggestions to improve this manuscript.

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



















313 **Box 1. Comparing risks of biopesticide resistance evolution**

314 Biopesticides include a wide range of active ingredients that differ substantially in mode of
315 action and the biochemical complexity of their interactions with pests. These differences have
316 important consequences for the risk of pests evolving resistance. In Figure I below, we
317 organise control methods from biochemically simple (at the left) to biochemically complex
318 (at the right), placing biopesticides (white columns) in the context of other crop protection
319 approaches (grey columns). The first three table rows present the existence of evidence for
320 insect resistance (in the field [3,13,15,16,50,51]; in laboratory selection studies [38,51–56];
321 and standing genetic variation in insect populations that selection could act on [51,57–64]).
322 Next, we present a general estimate of complexity of genetic architecture of resistance to
323 different pest control measures. We emphasise that for commercially produced biocontrol
324 agents, coevolution with the pest does not occur and therefore the genotype of the biocontrol
325 organism remains relatively constant. Finally, we present our synthesis of the overall risk of
326 pest resistance evolution for each crop protection technology. Resistance to biological agents
327 is usually not a binary condition. Instead, due to the frequently polygenic nature of resistance,
328 insect populations often tend to display a relatively continuous distribution of susceptibilities
329 across different genotypes.

330

331

332

	 Synthetic Chemicals	 Plant incorporated protectants	 Molecules derived from organisms	 Viral biopesticides	 Bacterial biopesticides	 Fungal biopesticides	 Nematodes	 Parasitoids (inundative release)	 Predators (inundative release)	 Classical & conservation biocontrol
Insecticidal product containing	Molecules with insecticidal properties. e.g., Pyrethroids	Gene from <i>Bacillus thuringiensis</i> transferred to a plant to express crystal-forming proteins (Cry toxins) produced by the bacterium	Substances found in living organisms e.g., <i>S. spinosa</i>	Viruses e.g., Baculovirus	Bacteria and their toxins e.g., <i>Bacillus thuringiensis</i>	Fungal spores e.g., <i>Metarhizium anisopliae</i>	Nematodes e.g., <i>Steinernema feltiae</i>	Insects that are parasitoids of the host pest's eggs, nymphs, or pupae	Arthropods that kill and feed on several to many individual prey during their lifetimes	Integrating beneficial natural enemies back into crop systems for pest control
Example	Deltamethrin	Bt cotton	Spinosad	<i>Cydia pomonella</i> granulovirus	Dipel	Green Muscle	Nemasys	Parasitoid wasp (<i>Encarsia formosa</i>)	Predatory mite (<i>Phytoseiulus persimilis</i>)	Vedalia beetle (<i>Rodolia cardinalis</i>)
Evidence of field resistance evolution	✓	✓	✓	✓	✓	*	*	*	*	✓
Evidence of resistance evolution during laboratory selection studies	✓	✓	✓	✓	✓	✓	*	✓	*	*
Evidence of genetic variation in susceptibility in natural populations	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Likely number of loci underpinning resistance	FEW	FEW to SOME	FEW to SOME	FEW to SEVERAL	SEVERAL	SEVERAL	SEVERAL	FEW to SEVERAL	SEVERAL*	SEVERAL*
Could natural enemy coevolution help prevent resistance?	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓
Risk of resistance (without appropriate management)										

333

334

335 Figure I. The biocontrol continuum of resistance risks. Ticks denote the existence of evidence
 336 for a given factor; ★ indicates the absence of evidence, Speedometer dials show risks of
 337 resistance evolution (red = high – green = low).

338

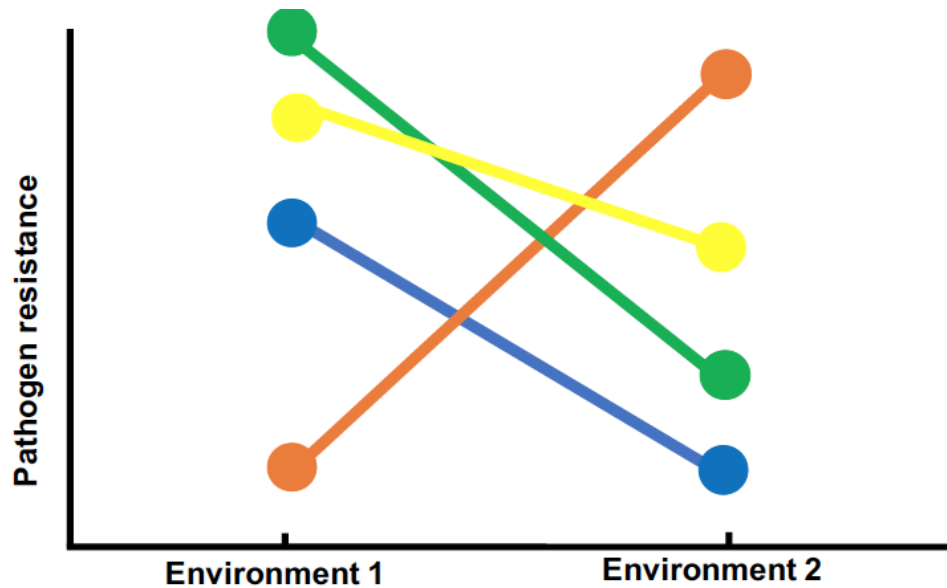
339 Agents at the left-hand side of the figure have relatively simple molecular interactions with
 340 target pests and are at greater risk of eliciting resistance evolution than those agents towards
 341 the right. Biopesticides based on **molecules derived from organisms** (e.g., spinosad) may
 342 not require meaningfully more complex resistance mechanisms than synthetic pesticides [17],
 343 and therefore, any selection can rapidly drive heightened resistance. Insect resistance to
 344 viruses can sometimes be based principally on just one or two loci [65–67], whereas for other
 345 viruses more genes are implicated [61,68]. Studies of resistance to bacterial and fungal
 346 infection in insects generally suggest a more complex genetic basis that can involve in excess
 347 of ten gene loci [22,62,69]. One can imagine that resistance to macro-parasites such as
 348 nematodes and parasitoids is probably also generally genetically complex; nevertheless, the
 349 few existing studies have suggested simple genetics underpinning resistance [63,70,71]. Even
 350 for predators, the genetics of resistance evolution can sometimes involve few genes of major
 351 effect, as illustrated by the famous case of industrial melanism in British peppered moths
 352 (*Biston betularia*) [72]. Studies of the genetics of resistance are undertaken under simplified

353 laboratory conditions and will therefore underestimate the number of loci involved in the
354 field. Whilst clearly there is variation among different classes of biological enemies, on
355 average we contend that the genetic basis of resistance is generally more complex than for
356 synthetic insecticides. Therefore, the greater number of genes involved in resisting attack
357 should make directional resistance evolution more difficult, because coadapted gene
358 complexes tend to be broken up every time meiosis occurs [73].
359

360 **Box 2. Genotype by environment interactions for pathogen resistance**

361 There is widespread evidence that the ability of any one genotype to defend against pathogen
362 infection depends on environmental parameters [31]. Thus, the most effective genotype for
363 parasite defence in one environment may not be the optimal genotype to survive infection for
364 hosts exposed to a different set of environmental conditions (Figure II). This change in the
365 relative fitness of resistance genotypes **is a genotype by environment interaction (GEI)**
366 [74]. For example, the optimum host genotype for pathogen defence often depends on the
367 species of pathogen [75] or on the genetic strain of a given pathogen species [76]. Another
368 major driver of GEIs for pathogen resistance in ectotherms is environmental temperature,
369 where individual genotypes are best able to defend against infection only over a specific
370 range of temperatures [77,78]. Furthermore, the relative ability of host genotypes to defend
371 against infection can strongly vary between different host diets [79]. Nevertheless, the ability
372 of environmental variables such as host diet and temperature to drive GEIs is apparently not
373 universal [80,81]. A further cause of inconsistent selection on host resistance genotypes is
374 that fitness conferred by a particular host genotype can be dependent on the presence and
375 genetic identity of symbiotic microbes within the host [82] and may also be influenced by
376 coinfection of the host by other pathogens [83]. Manipulation of variables such as these in
377 agricultural landscapes could be used to manage the threats of resistance evolution to
378 microbial biopesticides used for crop protection.

379



380

381 Figure II. Four host genotypes are shown by different coloured lines; the resistance rank
 382 order of the genotypes varies between the two environments making selection for pathogen
 383 resistance inconsistent.

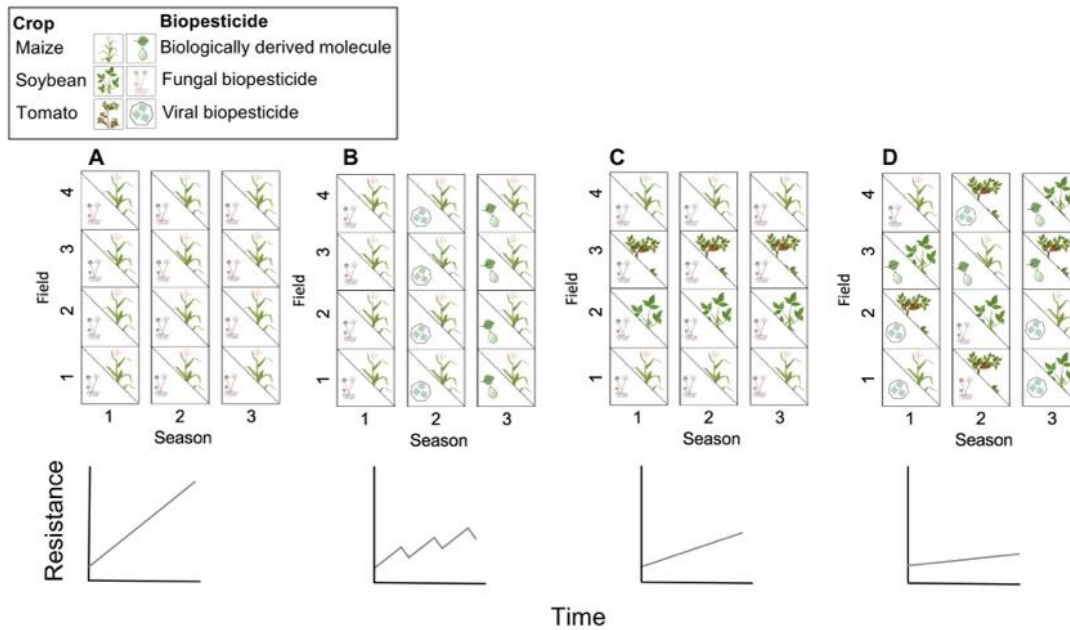
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385 **Box 3. Options for managing biopesticide resistance in agricultural landscapes.**

386 Applying single biopesticides consistently across large areas of the agricultural landscape that
 387 are ecologically homogenous heightens the risk of resistance evolution (Fig IIIA). Our vision
 388 is to strategically exploit GEIs to prevent uniform selection in biopesticide control
 389 technologies, thereby managing resistance to preserve the efficacy of these products for the
 390 long term. These GEIs could be generated by temporal (Fig IIIB) or spatial (Fig IIIC)
 391 heterogeneity, either in crop plants grown or biopesticides applied. However, the strength of
 392 GEIs to make selection for resistance inconsistent to mitigate resistance evolution risks is
 393 likely to be maximized if biopesticide identity and agricultural landscape variables (e.g., crop
 394 plant) are diversified simultaneously (Fig IIID).

395

396



397

398

399 Figure III. Four landscape scenarios for biopesticide use across three growing seasons, with
 400 three alternative biopesticides and three alternative crops. Spatial variation is shown along a
 401 vertical axis for four fields, whereas temporal variation is shown across three seasons

402 presented in a sequence horizontally in each case. The crop sown in each field is illustrated in
 403 the upper diagonal, while the biopesticide used is denoted in the lower diagonal. The

404 predicted trajectory of resistance evolution for each scenario is illustrated in the plots below

405 each scenario, based on presumed trade-offs across the landscape and the frequency of the
 406 focal combination of crop and biopesticide, which dictates the landscape-wide intensity and

407 directionality of selection. A) Homogeneous landscape e.g., static crop & pesticide use; B)

408 Temporally heterogeneous landscape e.g., pesticide rotation; C) Spatially heterogeneous

409 landscape e.g., crop mosaic; and D) Combined heterogeneous landscape e.g., **shifting**

410 **mosaic.**

411

412 **Glossary:**

413 **Biological control/biocontrol:** The use of living organisms or substances derived from them
 414 to attack target pest species.

415 **Biopesticides:** Pest control products formulated from living organisms or naturally occurring
 416 molecules derived from organisms. There are three branches of biopesticides: 1) Biochemical
 417 pesticides, based on naturally occurring materials, including pheromones, plant extracts/oils,
 418 or natural insect growth regulators, 2) Microbial biopesticides (see below) and 3) Transgenic
 419 crops (see below).

420 **Genotype-by-environment interactions:** A phenomenon in which the fitness of alleles
421 depends on the environment in which those alleles are expressed.

422 **Gene flow:** The introduction of new genetic material from one population to another through
423 dispersal.

424 **Integrated pest management:** An ecosystem-based strategy for long-term crop damage
425 reduction through techniques that seek to minimise economic and environmental risks.

426 **Microbial biopesticides:** Pesticides containing living microorganisms (viruses, fungi,
427 bacteria, or nematodes) as the active ingredient in the formulation.

428 **Mode of action:** The mechanism by which a pesticide attacks a pest. For synthetic pesticides,
429 this is frequently one or a few target biomolecules, while for living biopesticides, there may
430 be many modes of action that are not as easy to describe simply in biochemical terms.

431 **Molecules derived from organisms:** The use of naturally occurring products e.g.,
432 pheromones, plant extracts/oils, or natural insect growth regulators, to control pests.

433 **Pesticide resistance:** An increased probability of pest survival and reproduction in the face
434 of crop protection methods. To the extent that such abilities are heritable, repeated exposure
435 to pesticides can lead to evolutionary changes in pest populations that collectively cause
436 failures to achieve the expected level of control.

437 **Pesticide rotation:** The alternating use of different pesticides (with distinct modes of action)
438 in different areas of a farm or at different times to control target pests, in such a way that the
439 pest population is not constantly exposed to the same pesticide.

440 **Polyphagous pests:** Pests that can feed on crops belonging to many diverse taxonomic
441 groups.

442 **Red Queen Dynamics:** Continuous coevolutionary arms race between hosts and parasites,
443 whereby parasites evolve to be more infectious to hosts and hosts evolve to keep pace in their
444 ability to defend against infection. This process maintains genetic diversity for defence and
445 attack genes in the host and parasite population respectively.

446 **Refuge:** An area of crops on a farm in which no pesticides are applied, serving to weaken
447 selection for pesticide-resistance in pests and to serve as a reservoir for pesticide-susceptible
448 alleles.

449 **Resistance evolution:** Improvement over time in the genetic propensity of a pest population
450 to cope with pest control measures after repeated exposure to the control agent.

451 **Parasitoids:** Insects that use an insect host to develop, resulting in the death of the host.

452 **Shifting mosaic:** the sowing of alternating crop species through both space and time in a
453 local area, in such a way that the landscape is both spatially and temporally diversified.

454 **Spatial rotations:** Alternating the sequence of a crop grown or a pesticide applied in
455 different areas of a landscape.

456 **Temporal rotations:** Alternating the sequence of a crop grown or a pesticide applied at
457 different times in a given location.

458 **Transgenic crops:** Genetically modified plants that produce pesticides within their own
459 tissues, e.g., Bt cotton is engineered to contain and express one or more *B. thuringiensis*
460 insecticidal crystal proteins (e.g. Cry toxins) to prevent insect pest damage.

461

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