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A mathematical modeling approach to assess biological control of an orange tree disease

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Abstract

The model presented and investigated here describes the interaction between the orange tree and two different microorganisms, the pathogen fungus *Guignardia citricarpa* and the antagonist *Trichoderma harzianum T1A*. The pathogen-free and coexistence are the only possible system's equilibria. The pathogen-free points bifurcates from coexistence when the antagonist strength is sufficiently high, but does not appear to much be dependent from the amount of beneficial fungus employed. This result represents a relevant guideline for the applied ecologist and for the farmers. Sensitivity analysis in suitable parameter spaces is performed numerically.

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Orange tree, *Guignardia citricarpa*, *Trichoderma harzianum T1A*, mathematical model

1. Introduction

Citrus Black Spot, caused by the fungus *Phylosticta citricarpa* (previously known as *Guignardia citricarpa*), is one of the most damaging fungal citrus diseases causing significant yield losses in countries like Brazil, Australia and South Africa [23]. Citrus Black Spot is a foliar and fruit disease, characterised by hard spots, virulent spots, false melanose, freckle spots on fruit, and necrotic lesions on leaves and twigs [12]. It affects all commercial citrus cultivars, with injured fruits being commercially undeserved *in natura*, intended only for juice production.

Citrus Black Spot disease was reported for the first time in Australia [3], in 1895, and is currently present in warm, summer rainfall areas of Asia, Africa, South America and North America. Although Citrus Black Spot has never been reported in Europe, the fungus has recently been reported in several European countries (Italy, Malta and Portugal) [11], and is considered to have potential for establishment and spread [5].

Citrus fruits with Citrus Black Spot lesions are subject to quarantine regulations in the European Union [6] and USA [25]. The regulations restrict market access where fruit is quarantined; the fruit cannot be sold in the fresh market [14] thereby reducing the availability of citrus fruits to consumers in the off-season in Europe [1].

The control of Citrus Black Spot is exerted by applying fungicides, and the fruits need to be sprayed four to five times during the infection period [24]. In severe attacks, *P. citricarpa* can cause premature fruit fall, reducing plant productivity, with losses up to 80% in productivity. Fungicides have also been associated with negative environmental impacts [9] and cause the selection of strains resistant to the active principles used [10]. Biological control agents (BCA) have received large attention, and their use has contributed to the control of fungal diseases both as a complement or replacement of agrochemicals, with consequently lower ecological and economical costs. Biological control of pathogens relying on the use of living organisms to keep in check pests has been used for centuries [21]. *Trichoderma* species have been used with success as biocontrol agents [19] against numerous pathogens including *P. citricarpa* [16, 17, 15, 8, 4]. *Trichoderma harzianum* T1A secretome inhibits the growth and mycelial melanization of *P. citricarpa*. The biocontrol agent secretes proteins related to the control of *P. citricarpa*, and induction of plant resistance, even in the absence of pathogen challenge [17].

The objective of this investigation is to explore and evaluate the effectiveness of such biological practice through a mathematical model. We describe the behaviour of a biological system composed by an orange tree, the pathogen fungus, *Guignardia citricarpa*, and the beneficial one, *Trichoderma harzianum T1A*. In the next Section we formulate the model, which is qualitatively analysed in Section 3. Bifurcations are investigated next. Some numerical simulations support the theoretical findings and indicate possible management strategies. The results are discussed in Section 6.

2. The model

The three populations of interest here are the orange tree fruits, O, the pathogen fungus *G. citricarpa*, P, and the beneficial fungus *T. harzianum T1A*, F. The model reads:

$$\begin{aligned} \frac{dO}{dt} &= rO\left(1-\frac{O}{K}\right) - h(F)OP, \\ \frac{dP}{dt} &= eh(F)OP - cP^2 - aFP, \qquad\qquad h(F) = \frac{1}{q+F}, \\ \frac{dF}{dt} &= sF\left(1-\frac{F}{H}\right) + baFP. \end{aligned} \tag{1}$$

The first equation describes the evolution of the orange tree, growing logistically with net growth rate r and carrying capacity K, being negatively affected by the pathogen fungus. This however is somewhat reduced by the presence of F and modeled via the dependence on the interaction coefficient on F. The larger the amount of F, the most effective the reduction is, so that we take the variable parameter $h(F)$ to be a decreasing function.

The second equation contains the evolution of the pathogen fungus feeding on the tree parts, where $e < 1$ represents a conversion factor. Intraspecific competition for resources at rate c is taken into account. The fungus cell walls are degraded by the extracellular enzymes produced by *Trichoderma* at rate a for which *G. citricarpa* experiences a loss.

In the third equation the beneficial fungus feeds on resources that are not explicitly modeled, logistic term with net reproduction rate s and carrying capacity H, but also obtains additional food by degrading the bad fungi with conversion coefficient $b < 1$.

3. The qualitative analysis of the model

3.1. Boundedness

In order to have a well-posed model, we show that the system's trajectories remain confined within a compact set. Consider the total system population $\varphi(t) := O(t) + P(t) + F(t)$. Summing up the equations of (1) for an arbitrary $\eta > 0$, dropping the negative terms, since $e < 1$ and $b < 1$, we then have

$$\frac{d\varphi(t)}{dt} + \eta\varphi(t) = \Pi(O) + \chi(P) + \Gamma(F) \leq \Pi_M(O) + \chi_M(P) + \Gamma_M(F)$$

$$\Pi(O) = (r+\eta)O - \frac{r}{K}O^2, \quad \chi(P) = (\eta - cP)P, \quad \Gamma(F) = (s+\eta)F - \frac{s}{H}F^2$$

$$\Pi_M = \Pi(O_M) = \frac{K(r+\eta)^2}{4r}, \quad O_M = \frac{r+\eta}{2r},$$

$$\chi_M = \chi(P_M) = \frac{\eta^2}{4c}, \quad P_M = \frac{\eta}{2c},$$

$$\Gamma_M = \Gamma(F_M) = \frac{H(s+\eta)^2}{4s}, \quad F_M = \frac{s+\eta}{2s}.$$

Setting $M = \min\{\Pi_M, \chi_M, \Gamma_M\}$ and dropping the negative terms, we have the differential inequality $\varphi'(t) \leq M\varphi(t)$ from which, upon its solution, the upper bound follows

$$\varphi(t) \leq \max\{M, \varphi(0)\}.$$

Since each population is nonnegative, it is bounded by the same upper bound as well.

3.2. System's equilibria

System (1) has seven possible equilibria, the configuration $E_2 = (0, P_2, 0)$ not being allowed because, biologically, P is a specialist predator on the orange tree and in its absence, it cannot thrive. Also $E_6 = (0, P_6, F_6)$ is unfeasible, as it is easily checked that $F_6 < 0$.

For stability assessment, the Jacobian of (1) is needed:

$$J = \begin{bmatrix} r - \dfrac{2rO}{K} - \dfrac{P}{q+F} & -\dfrac{O}{q+F} & \dfrac{OP}{(q+F)^2} \\ \dfrac{eP}{q+F} & \dfrac{eO}{q+F} - 2cP - aF & -\dfrac{eOP}{(q+F)^2} - aP \\ 0 & baF & baP + s - \dfrac{2sF}{H} \end{bmatrix}. \quad (2)$$

Equilibria $E_0 = (0,0,0)$, $E_1 = (K,0,0)$ and $E_3 = (0,0,H)$, all unconditionally feasible, are all unstable. Indeed for E_0 the eigenvalues are r, 0 and s; for E_1 they are $-r$, eKq^{-1} and s; for E_3 we find r, $-aH$, $-s$. Further, $E_5 = (O_5, P_5, 0)$ with

$$O_5 = \frac{rq^2K}{rq^2 + ceK}, \quad P_5 = \frac{ce}{q} O_5$$

is also always feasible but unstable by the positive eigenvalue $s + abP_5$.

For the pathogen fungus-free equilibrium $E_4 = (K,0,H)$, E_4 finally the eigenvalues are $\lambda_1 = -r$, $\lambda_2 = [eK - aH(q+H)](q+H)^{-1}$ and $\lambda_3 = -s$ giving the stability condition

$$eK < aH(q+H). \quad (3)$$

For coexistence, $E_* = (O_*, P_*, F_*)$, solving the third equation of (1) and substituting it into the first one, we get

$$P_* = \frac{sF_* - Hs}{Hba}, \quad O_* = \frac{HKbarq + HKs + F_*(HKbar - Ks)}{Hbar(rq + eF_*)} \quad (4)$$

where F_* is a real positive root of the cubic $\Psi(F) := AF^3 + BF^2 + CF + D = 0$ with $A = (Ha^2b + cs)r > 0$, $D = -H[esK + qr(Kabe + cqs)] < 0$, which ensure at least one positive root, and

$$B = [((a^2bq - cs)H + cqs + q(Ha^2b + cs)]r \quad (5)$$
$$C = [((a^2bq - cs)H + cqs)q - H(Kabe + cqs)]r - sKe.$$

However, nonnegativity of O_* and P_* must be ensured. It follows from

$$\frac{s - Hbar}{s + barq} < \frac{H}{F_*} < 1. \quad (6)$$

The Routh-Hurwitz stability conditions in this case are

$$-\mathrm{tr}(J(E_*)) = \frac{r}{K}O_* + cP_* + sF_* > 0,$$

which holds, and letting $M^{(2)}$ be the sum of the minors of order two of $J(E_*)$,

$$M^{(2)} = \frac{r}{K}O_*P_* + \frac{eP_*O_*}{(q+F_*)^2} + s\frac{r}{K}F_*O_* + csF_*P_* + abF_*\left(a + \frac{eO_*}{(q+F_*)^2}\right)P_*,$$
$$\det(J(E_*)) = F_*O_*P_*\left[\frac{crs}{K} + \frac{abeP_*}{(q+F_*)^3} - \frac{abr}{K}\left(a + \frac{eO_*}{(q+F_*)^2}\right) - \frac{es}{(q+F_*)^2}\right],$$

so that the remaining stability conditions are given by

$$\det(J(E_*)) < 0, \quad \mathrm{tr}(J(E_*))M^{(2)} < \det(J(E_*)), \tag{7}$$

which we avoid to write down explicitly.

Table 1 summarizes feasibility and stability of the system's equilibria.

Table 1: Characterization of the equilibria of (1).

$E = (O, P, F)$	Feasibility conditions	Stability conditions
$E_0 = (0, 0, 0)$	—	unstable
$E_1 = (K, 0, 0)$	—	unstable
$E_2 = (0, P, 0)$	unfeasible	—
$E_3 = (0, 0, H)$	—	unstable
$E_4 = (K, 0, H)$	—	(3)
$E_5 = (O_5, P_5, 0)$	—	unstable
$E_6 = (0, P_6, F_6)$	unfeasible	—
$E_* = (O_*, P_*, F_*)$	(6)	(7)

4. Bifurcation analysis

Concentrating on the pathogenic fungus-free equilibrium, we observe that the eigenvalue λ_2 vanishes for the parameter choice

$$a^\dagger = \frac{eK}{H(q+H)}. \tag{8}$$

The Jacobian evaluated at E_4 simplifies to

$$J = \begin{bmatrix} -r & -\dfrac{K}{q+H} & 0 \\ 0 & 0 & 0 \\ 0 & abH & -s \end{bmatrix}$$

for which the right and left eigenvectors respectively are $\mathbf{v} = (v_1, 1, v_3)^T$, with $v_1 = -K[r(q+H)]^{-1}$, $v_3 = abHs^{-1}$, and $\mathbf{w} = (0, 1, 0)^T$. Also, denoting by $\mathbf{f}(O, P, F) = (\mathbf{f}^1, \mathbf{f}^2, \mathbf{f}^3)$ the right hand side of (1), and by subscripts the partial derivatives, we find

$$\mathbf{f}_a = \begin{pmatrix} 0 \\ -PF \\ bPF \end{pmatrix} \quad D\mathbf{f}_a = \begin{bmatrix} 0 & 0 & 0 \\ 0 & -F & -P \\ 0 & bF & bP \end{bmatrix}, \tag{9}$$

from which $\mathbf{f}_a(E_4, a^\dagger) = \mathbf{0}$ and consequently $\mathbf{w}^T \mathbf{f}_a(E_4, a^\dagger) = 0$. This result represents the first condition in Sotomayor's theorem, [22], that we use at present. Further, $\mathbf{w}^T D\mathbf{f}_a(E_4, a^\dagger)\mathbf{v} = -H \neq 0$, the second required condition for the existence of bifurcations. We then need to evaluate

$$\mathbf{w}^T D^2\mathbf{f}(E_4, a^\dagger)(\mathbf{v}, \mathbf{v}) = D^2\mathbf{f}^2(E_4, a^\dagger)(\mathbf{v}, \mathbf{v}).$$

Observe that the second partial derivatives of $\mathbf{f}^2$ are $\mathbf{f}^2_{OO} = 0$, $\mathbf{f}^2_{PP} = -2c$ and

$$\mathbf{f}^2_{OP} = \frac{e}{q+F}, \quad \mathbf{f}^2_{OF} = -\frac{eP}{(q+F)^2}, \quad \mathbf{f}^2_{PF} = -a - \frac{eO}{(q+F)^2}, \quad \mathbf{f}^2_{FF} = \frac{2eOP}{(q+F)^3}$$

and thus at this equilibrium all vanish, except three. In summary, we find

$$\begin{aligned} D^2\mathbf{f}^2(E_4, a^\dagger)(\mathbf{v}, \mathbf{v}) &= 2\frac{e}{q+F_4}v_1 - 2c - 2\left(a^\dagger + \frac{eO_4}{(q+F_4)^2}\right)v_3 \\ &= -2\frac{eK}{r(q+H)^2} - 2c - 2\frac{a^\dagger bH}{s}\left[a^\dagger + \frac{eK}{(q+H)^2}\right] \\ &= -2eK\left[\frac{1}{r(q+H)^2} + \frac{c}{eK} + \frac{q+2H}{(q+H)^3}\frac{beK}{sH}\right] \neq 0. \end{aligned}$$

so that also the third condition for the occurrence of a transcritical bifurcation is satisfied. Since in this case when a crosses from above the critical threshold $a^\dagger$ the equilibrium E_4 becomes unstable and the P population appears in the system, the latter settles to the coexistence equilibrium and the pathogen establishes permanently in the system.

5. Numerical simulations and discussion

The findings of the previous section are also supported by numerical experiments. Figure 1 contains the bifurcation diagram of O, P, F in terms of the degradation rate a of P by F.

(a) For $a = 0.1$ a transcritical bifurcation arises between the coexistence equilibrium and the pathogen fungus-free equilibrium, E_4.

(b) For $a < 0.1$ an interesting behaviour occurs. Increasing a increments the density of the beneficial fungus but when the degradation rate a attains a threshold, here 0.05, the beneficial fungus experiences a decline in the reward gotten from the action of the pathogen fungus, because the density of the latter becomes too low.

(c) For $a > 0.1$ the system (1) attains the pathogen-free point, the most important ecological equilibrium, with both O and F at their respective carrying capacities K and H. Note that the equilibrium populations at E_4 do not depend on a, that no change in their values occurs by increasing a.

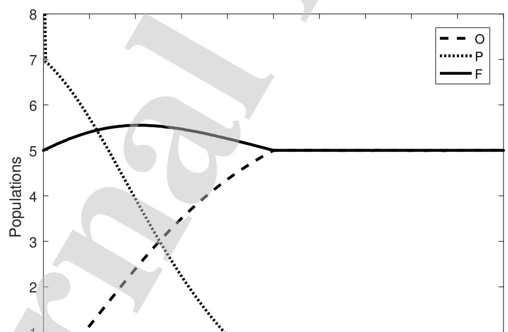


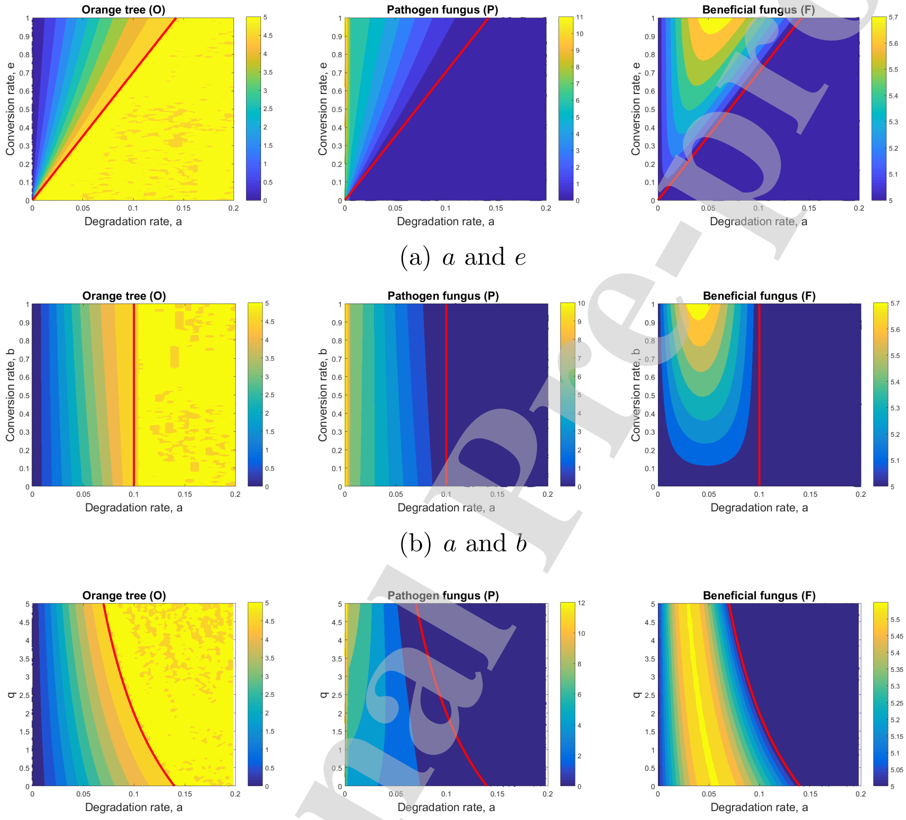
Figure 1: The bifurcation diagram of O (dashed), P (dotted) and F (continuous line) with respect to the degradation rate of P by F, a. The other parameters values are $r = 1$, $K = 5$, $e = 0.7$, $b = 0.7$, $s = 1$, $H = 5$, $q = 2$ and the i.c. are $(1, 1, 1)$. With these parameter values it can easily be checked that $a^{\dagger} = 0.1$ coinciding with the value expected from (8), so that a transcritical bifurcation arises between E^* and E_4.

Figure 2 shows the densities of the populations O, P and F, at steady state, in terms of two model parameters: (a, e) in panel (a); (a, b) in panel (b); (a, q) in panel (c). In all these situations by fixing the value of the parameter on the $y-$axis and increasing a leads to an increase in the density of O, in an decrease in the density of P, while for F at the beginning there is an increase followed by a decrease. This latter feature corresponds to what is observed in Figure 1. Let us now fix the value of a. In panel (a) we find that increasing the parameter e, both P and F increase, while O decreases. In panel (b) increasing b, O experiences a slow increase, P has a slow decrease and F increases. In panel (c) increasing q, both O and P increase while F first increases and then decreases.

The biological control of Citrus Black Spot has been shown but only by scarce studies. Among them, the use of *Saccharomyces cerevisae* Meyer [20, 7, 8] and the possibility of using volatile organic compounds produced by yeast was reported. Also, bacteria of the genus *Bacillus*, such as *B. thuringiensis* var. *kurstaki* (HD-1), obtained from the commercial products Dipel®WP and Dimy Pel®, and *B. thuringiensis* var. *kurstaki* (HD-567), used in the control of *P. citricarpa*, demonstrated to reduce the number of picnids per *P. citricarpa* lesion, and the number of lesions per fruit [18].

Guimarães, [13] used as an antagonist the fungus *Trichoderma koningii* isolated from the surface of "*Montenegrina*" tangerine leaves. The authors were able to control *P. citricarpa* in vitro and in vivo using the same orchard from where the antagonist was isolated. It has also been demonstrated that *T. koningii* reduces the severity and incidence of Citrus Black Spot.

Other organisms or combinations of microorganisms may have different efficiencies when compared to experiments under field conditions. [4] used *T. harzianum*, *B. subtilis* and a biofertilizer, which had a microbial load composed mainly of bacteria (*Bacillus* spp, *Pseudomonas* spp and actinobacteria) to control *P. citricarpa* in citrus fruits. Best results were achieved with the biofertilizer, followed by *B. subtilis* and *Trichoderma*. As pointed out by [15], antagonism of fungal pathogens under laboratory conditions may not be reflected under field conditions. The authors observed that *Bacillus subtilis* was able to control *P. citricarpa* in vitro, but the efficiency under field was not reproducible, requiring further studies to select more efficient isolates and the best application period.



(a) a and e

(b) a and b

(c) a and q

Figure 2: The density of O, P and F at stable state, as function of two parameters. The red curve, obtained by (3), partitions the domain into the stable coexistence equilibrium (on its left) and the pathogen-free point E_4 (on its right). Note that the axes of the colorbars are different in each figure.

6. Conclusions

Recent studies [16, 17] show that *T. harzianum* T1A and *Trichoderma atroviride* T17 are able to control *Guignardia citricarpa* Gc3. Unlike other *T. harzianum* strains, T1A is able to induce plant resistance without being challenged by a pathogen.

Mathematical analysis is instrumental in defining strategies for the biological control of this infestant. Policies to possibly use *Trichoderma harzianum T1A* in orange cultures to fight *Guignardia citricarpa* and reduce farmers' economic losses can be devised on this analysis.

The system has two possible equilibria, the pathogen fungus-free point and coexistence. Condition (8) states that from the latter the system settles to the former if the degradation rate of fungus cell walls a by *Trichoderma harzianum T1A* falls below the critical threshold $a^{\dagger}$. In such case the pathogen *Guignardia citricarpa* becomes endemic. However, note that annihilating λ_2 for the pathogen-free equilibrium E_4 can be obtained also by parameter choices other than a, (8). For instance, one can choose $e^{\dagger} = aH(q+H)K^{-1}$. In this case it is easily checked that both $\mathbf{w}^T\mathbf{f}_e(E_4, e^{\dagger}) = 0$ and $\mathbf{w}^T D\mathbf{f}_e(E_4, e^{\dagger})\mathbf{v} = K(1+H)^{-1} \neq 0$ still hold. As no change in the second derivatives $D^2\mathbf{f}$ occurs, the transcritical bifurcation arises also crossing the critical value $e^{\dagger}$. From an ecological point of view e is a conversion factor, that measures the efficiency on how the pathogen fungus is able to exploit the nutrients obtained from the orange fruits. When its value is 1, maximum efficiency is obtained. But, independently of the value, if e falls below the critical value $e^{\dagger}$, E_4, the pathogenic fungus-free point is stable, while if it lies above the threshold $e^{\dagger}$, E_4 becomes unstable. The system trajectories then move to the only other possible allowed stable equilibrium, E_*. This change in the ultimate behavior of the system represents the meaning of a transcritical bifurcation. The coexistence equilibrium E_* contains all the populations, and therefore the pathogen *Guignardia citricarpa* invades the ecosystem and becomes endemic in it. To remove the pathogenic fungus or keep the system pathogen-free, the conversion factor should be less than the threshold, $e < e^{\dagger}$. Because the conversion factor is an intrinsic property of the fungus, it is hard to be altered by human actions. However, the value of the threshold can be raised, to enhance the satisfaction of the inequality. This could be achieved if the carrying capacity K of the orange tree is lowered, or conversely the carrying capacity H of the beneficial fungus is raised. These measures can likely be more easily implemented by the farmers, especially for instance by pruning the orange tree. Note that the same measures have the opposite effect on the degradation rate's a critical threshold $a^\dagger$, of the fungus cell walls, lowering it, but with the ultimate same effect on the ecosystem.

Instead, attempting to use H as bifurcation parameter, for which the critical value would be obtained by the positive root of the quadratic $aH^2+aqH-eK=0$, namely $H^\dagger=(2a)^{-1}[\sqrt{a^2q^2+4aeK}-aq]$, leads to $\mathbf{w}^T\mathbf{f}_H(E_4,H^\dagger)=0$ as well as $\mathbf{w}^T D\mathbf{f}_H(E_4,H^\dagger)\mathbf{v}=0$, for which no bifurcation can arise. Similarly, taking $K^\dagger=aH(q+H)e^{-1}$, it follows $\mathbf{w}^T\mathbf{f}_K(E_4,K^\dagger)=0$ as well as $\mathbf{w}^T D\mathbf{f}_K(E_4,K^\dagger)\mathbf{v}=0$ and again no bifurcation can arise. This entails that the measures discussed above on pruning the trees, thus lowering K, or fostering the *Trichoderma harzianum T1A*, cannot alone directly lead to a transcritical bifurcation and consequent *Guignardia citricarpa* eradication. Nevertheless, as they lead to favorable changes in the thresholds $e^\dagger$ and $a^\dagger$, their exploitation combined with perhaps small modifications either in the conversion factor e or the degradation rate a could help for the pathogen eradication.

We can conclude that these remarks are important for the ecologist working in the field, and constitute a guideline for possible economic advantages for the farmer. Indeed, they hint that to eradicate the pest, the effectiveness of the antagonist here expressed by the parameters a and e, has more influence than the amount that is actually sprayed, represented in a sense by the fungus carrying capacity H, or alternatively by the size of the tree receiving the treatment, modeled via its "size" K.

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Conflict of interest The authors declare that they have no conflict of interest.

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