Can Lichens form a Barrier for Fungi Marching on?

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Abstract

The spread of new fungal diseases forms one of the major challenges in a changing planet. The rise of the Sudden Oak Death Pathogen *Phytophthora ramorum* has caused severe economic damage to large forested areas in the Northern hemisphere. In this paper, we present a review on the available data on this relatively new disease in the framework of global biodiversity impairment. The observation of the absence of *P. ramorum* in old, Lichen-covered forests adjacent to infected forests, was the starting point for a number of critical questions. For, it is also known that Lichens contain a substantial number of chemical compounds, some of which are known to interfere with important biochemical pathways in *Phytophthora* as well as in many other organisms. Moreover, mixed spatial and temporal bio-mathematical models are evaluated in order to find a manageable approach for predicting further spread of *P. ramorum* in threatened forest areas. Still a lot of questions remain unanswered and urge for further observation and data collection in the field.

Keywords: Biodiversity; Epidemiology of *Phytophthora ramorum* ; Lichens; Organic Inhibitory Substances; Mixed Spatio-Temporal Models for Spread of Infections

Introduction

During the last decade, a new exotic plant pathogen *Phytophthora ramorum* has caused an epidemic in the US and Europe, by killing whole groups of oak trees (*Quercus spp.*), tanoak (*Lithocarpus densiflora*) (Pacific coast) and Japanese larch (*Larix kaempferi*) (UK) in large areas of woodland [1,2]. The genus *Phythophtora* belongs to the class of the Oomycetes, a subdivision of the *Stramenophiles* [3](*). The *Oomycetes* are all heterotrophic, parasitic organisms, causing blights, mildews and molds. The recently discovered *P. ramorum* can infect a number of different trees and shrubs, including Douglas fir (*Pseudotsuga menziesii*), beech (*Fagus* spp.), oak, larch, *Rhododendron* and *Viburnum* [4,5]. *P. ramorum* provides an obvious example of the emergence of a pathogen via so-called human-assisted global migration [2]. Following several epidemic outbreaks in the US and Europe, national and regional authorities have ordered to contain the epidemic or slow down the progressive spread of the fungus by large scale tree felling.

(*) According to Nicholls (2004), *Phytophthora ramorum* is a pathogen that belongs to the kingdom (regnum) of the Stramenophiles, a taxon that is very similar to the kingdom of Fungi, but that is most closely related to the Brown algae and Diatoms. According to Campbell et al. (2015), the *Stramenophiles* indeed contain the taxa of Diatoms, Golden algae and Brown algae, and belong to the so-called 'SAR' clade, which is, however, a hypothetical phylogenetic taxonomy of the Eukaryotes, defined by whole-genome DNA sequence analysis. In this hypothetical scheme, several groups of organisms previously known as Protists, have now been brought together in new, hypothetical clades. *Stramenophiles* and *Fungi* would belong to very distinct clades in this scheme, despite the fact that both clades would contain 'slime molds'.

(see: Campbell NA, Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV and Jackson RB [2015, 10th Ed.], *Biology. A Global Approach*. Pearson, Boston, New York, Tokyo, pp. 652-657.)

Following closer observation in heavily infected areas in Central-Wales, we noted that in parts of the forests, adjacent to infected areas, trees covered with abundant vegetation of mosses and lichens apparently were not infected (Figure 1 A, B). An important question raised here – also for economic reasons - is whether the spread of

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Phytophthora ramorum, for instance due to airborne dispersal via asexual sporangia or via chlamydospores in the soil (Figure. 2) [3], through the influence of animals inhabiting the forest, or by walkers, mountain bikers or otherwise, through the loose mud on their clothing, shoes and bike tyres, could be slowed down by other means than by felling the trees? Shortly: can lichens form a barrier hampering the spread of this fungus-like pathogen?

(1A)

(1B)

Figure 1. A(top left). Large-scale larch tree (*Larix kaempferi*) removal in infected woodland in Central-Wales; B(below). Abundant coverage of mosses and lichens on beech (*Fagus sylvatica*) in non-infected woodland, adjacent to the area shown in A; A (middle) . Public warning signal in Central-Wales (A-C, © 2019, Biological Publishing A&O).
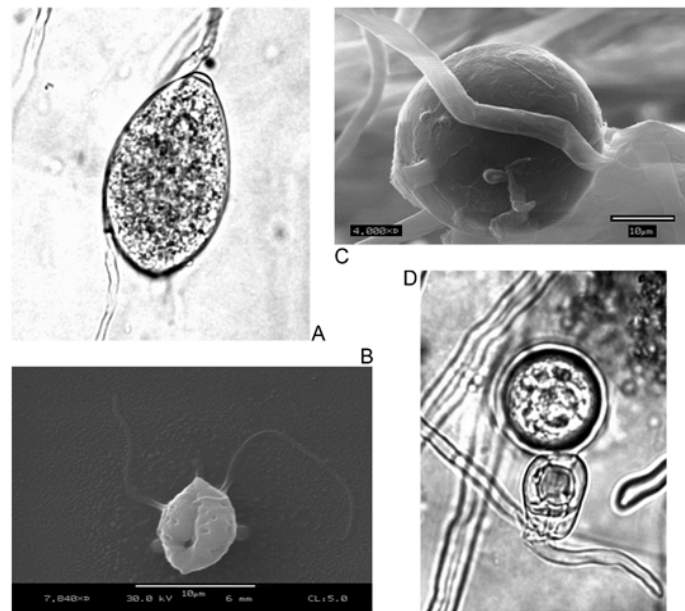
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Figure 2. Reproductive structures of *Phytophtora*: A. Asexual sporangia, B. asexual zoospores, C. asexual chlamydospores and D. sexual oospores (Reproduced courtesy of Matteo Garbelotto, UC Berkeley [A, D], and Edwin R. Florance, Lewis & Clark College, Portland, Oregon and the USDA Forest Service Pacific Southwest Research Station in Albany, California [B, C])(PLoS Biology, 2004).

Recently, it has been shown that the growth and activity of several *Phytophthora* species were severely inhibited by lichen extracts and lichenic acids in particular, like usnic acid and evernic acid (see 2. The Chemical Arsenal of Lichens) [6]. Lichens are symbiotic species consisting of lichen-forming fungi in combination with algae. Lichens are known to be extremely slow growing organisms, at most increasing only a few millimeters each year (Figure, 3A). On the other hand, some lichens are exceptionally long lived, resulting in reports of a life span of several hundreds or possibly even thousands of years [7]. Moreover, lichens form a well-known indication of the absence of air pollution, for instance certain species grow selectively in conditions where (mean winter) SO_2 is less than 35 µg m^{-3} [7]. Some of these rare species that are indicative for unpolluted air, namely the Old Man's Beard (*Usnea florida*) (Figure. 3B) and Reindeer Moss (*Cladonia sp*),

are also the lichens where usnic acid was first discovered and isolated from (see 2. The Chemical Arsenal of Lichens).But also far more common lichens may interact with fungal species in the forest.

Further in this paper we examine the alternative explanatory schemes that either no interaction occurs between lichens and *P. ramorum,* or, that (certain) lichens would promote the spread of *P. ramorum*, or, that in certain conditions (e.g. in deciduous forests) both groups depend on similar physical conditions (temperature, humidity, and wind-speed). So not only the complex biochemical interactions with lichenic substances are reviewed but also an outline is presented for a mixed model for studying the spread of *Phytophthora sp*. in heterogeneous spatial domains containing old lichen-covered forests and relatively young cultivated woodlands.

(3a)

(3b)

Figure 3. A (top). Typical slow-growing lichens (*Cladonia sp*.) in pristine forest in Montenegro-Albania border region. B (center). Old Man's Beard (*Usnea florida*), a marker species for unpolluted air, photographed in non-infected woodland, Central-Wales. C (top) Infected larch tree (*Laryx*) in woodland in the Netherlands. D (bottom) Detail of trunk of tree shown in C, revealing typical 'bleeding canker' (A-D, © 2019, Biological Publishing A&O).

The Chemical Arsenal of Lichens: A Putative Defense Mechanism Against *Fungi*

Already in the nineteenth century, the German chemist Wilhelm Knop (1817-1891) discovered some peculiar chemical produced by lichens, like usnic acid (see Figure. 4C), that recently gained a lot of interest in the medical world [8]. Usnic acid was already synthesized by Frank H. Curd and Alexander Robertson in 1933 [9]. Orsellinic acid (Figure. 4A) is an important cornerstone in the biochemistry of lichens, like *Parmelia* sp. from which it can be extracted [10]. Evernic acid or lecanoric acid methylesther (Figure. 4B), is also an inhibitor of the growth [6] of *Phytophthora infestans*, a well-known, dreaded 'fungal' disease in potatoes [3,11].
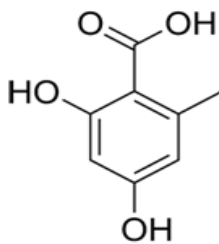
Figure 4A. Examples of chemical substances typically found in Lichens: A. Orsellinic acid (found e.g. in *Parmelia/Imbricaria latissima*)
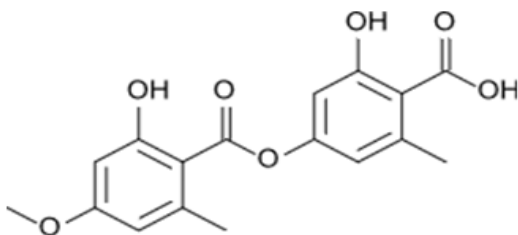

Figure 4B. Evernic acid, also known as lecanoric acid methylester (found in *Lecanora* sp.)
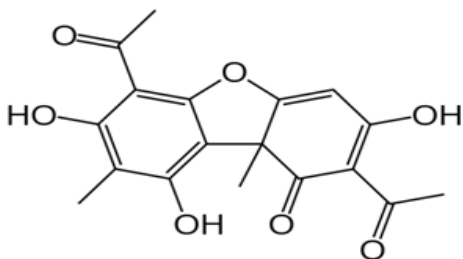

Figure 4C. Usnic acid (found e.g. in *Usnea* sp., *Cladonia* sp.)

Many of these lichenic substances belong to the groups of depsides and depsidones, which substances have recently been found to work as potent inhibitors of Microsomal Prostaglandin E2 Synthase-1 [8]. Depsides (Figure. 4D) are polyphenolic compounds especially found in lichens (but not exclusively) and generated from two or more monocyclic aromatic units linked by an ester bond, called the depside bond. Similarly, depsidones are cyclic ethers, generated by dimerization of phenolic acids like orsellinic acid via esterification (and subsequent oxidative biaryl ether formation) (Figure. 4E) [12]. Evernic acid is a methyl ester of a depside dimer of orsellinic acid. Similarly, usnic acid has a dibenzofuran structure derived from a dimerization of orsellinic acid. Some of these compounds may also become hazardous for some animals, as demonstrated in toxicity experiments with usnic acid in rats [13]. Not only prostaglandins play a role in the inhibition of (certain) cytochrome P 450 enzymes and physiological reactions to endotoxins [14], moreover, compounds of the furanocoumarin type

(Figure. 4F), found in several cultivated plants, appear to increase the toxicity of a variety of substances by inhibition of the cytochrome P 450 pathway [15].
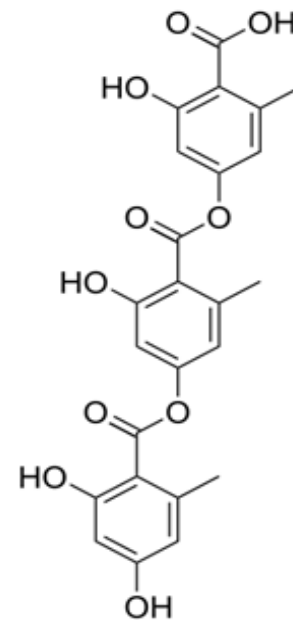

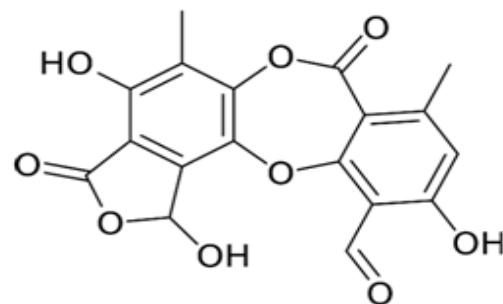**Figure 4D.** Example of depside: gyrophoric acid

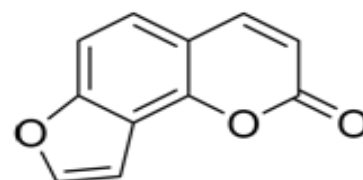
Figure 4E. Example of depsidone: norstictic acid

Figure 4F. Furanocoumarin, a different ester from cultivated plants, is also an inhibitor of cytochrome P-450 (References see main text; chemical structures from *Wikipedia, Chemistry*).

Not only it is important to investigate the role of chemical substances (in combination with other physical factors like humidity, temperature, air pollution, etc.) in the competitive interaction between different species of lichens and between lichens and fungus-like organisms. Although we don't know the specific role of inhibitors of the cytochrome P450 pathway in *P. ramorum*, in the first place because of the complex diversity of the P450 enzyme family in the *Oomycetes* class - and including the diversity of P450 in *P. ramorum* [16]–an ecological approach to study the interaction of lichens and *P. ramorum* offers an interesting perspective for further research. For the present paper, we assume that such interactions between lichens areat least very probable, for the well-known reason that different lichen species grow on quite distinct locations. Moreover, they are often mutually exclusive to a degree that the occurrence of certain lichenic species can be used as an indicator for certain biotopes or indicators for the zonation of biotopes such as e.g. rocky shores [6,17].

Epidemiology of the Spread of *Phytophthora ramorum*

Since the discovery of *Phythophtora ramorum* as a new fungus-like pathogen [1-5], the epidemic has rapidly spread in the temperate climate zones of the Northern hemisphere. Worldwide, however, the characteristics of this pandemic have revealed different targets of infection and different clonal lineages have been genetically analyzed [2]. Whereas initially, in the early nineties, the pathogen was discovered in Germany and the Netherlands on *Rhododendron* (and also on *Viburnum*) [2,5], several other groups of trees became infected during the following decade(s). In Northern America, the major impact of *P. ramorum* was reported for oak (*Quercus spp.*) and tanoak (*Lithocarpus densiflora*) from the coastal woodlands of California and southwestern Oregon [1,2] and the epidemic was designated as the Sudden Oak Death disease.

In Europe, and especially in Wales, Scotland, Northern Ireland and the Republic of Ireland, widespread tree mortality was predominantly observed in plantations of Japanese larch (*Larix kaempferi*), but also on hybrid (*Larix eurolepis*) and European larch (*Larix decidua*) [4]. However, many other conifer tree species, like Douglas fir (*Pseudotsuga menziesii*), true firs (*Abies grandis* and *A. procera*), western hemlock (*Tsuga heterophylla*) and Sitka spruce (*Picea sitchensis*) could all be infected when growing near the infected larch [4,18]. Also many deciduous tree species like beech (*Fagus sylvatica*), southern beech (*Nothofagus* sp.) and non-native oak species, including red oak (*Quercus rubra*), Turkey oak (*Q. cerris*) and Holm oak (*Q. ilex*) and also the horse chestnut (*Aeculus hippocastanum*) were discovered to become occasional hosts [18]. Since 2015, increasing numbers of European sweet chestnut (*Castanea sativa*) have been found affected in southern England [18].

On tree trunks, the *P. ramorum* infection is visible as typical lesions called 'bleeding cankers'. These lesions appear as black crusts; underneath the bark is discolored and dying. In contrast to the very susceptible larch woodlands, *P. ramorum* was found to be nonlethal for the infected Rhododendron. Economically, the devastations have been most detrimental to larch plantations, for instance in certain areas in South Wales where up to 78% of the trees were infected (counted during spring 2015) [4].

Although *P. ramorum* was first reported in the UK in 2002, the epidemic was not identified before 2010 in Wales. However, the disease has since spread across large areas, with almost a doubling of the infected area and number of trees in Wales in 2013 (from 3000 hectares to 6000 hectares, corresponding with around 3 million to 6 million infected trees) [4]. From a survey in March 2014, assisted by aerial views of discolored tree foliage, a much smaller increase (only 40 hectares, corresponding to 40 thousand trees) was estimated during the year 2014. Possibly, the exceptionally wet summer of 2012 provided the ideal conditions for the spores to spread, for it is known that high humidity conditions are favorable for the sporulation and spread of *P. ramorum* spores. Therefore, it is not surprising that in Europe, Ireland, Scotland and Wales where among the most vulnerable regions for the spread of this pandemic.

Other reasons for the special vulnerability of larch plantations, may result from the fact that larch is a pioneer species that needs relatively few nutrients [19]. Consequently, larch species are particularly light demanding and fast growing, the Japanese larch even exceeding the European larch in volume growth and growth speed [20]. In contrast to other conifers, the larch is deciduous, thus leaving a lot of leaf debris on the soil - as well as a rising soil temperature - which creates ideal conditions for survival of the (asexual) chlamydospores (Figure 2) of *P. ramorum* [2]. On the contrary, larch is less suitable for so-called 'Dauerwald' or continuous-cover silviculture [19]. To conclude, the introduction of Japanese larch in Europe in the second half of the nineteenth century, economically may not have reached its full exploitation potential [19].

However, serious economical drawbacks may have resulted from creating the ideal conditions for the spread of the *P. ramorum* epidemic, especially during the temperate and wet summer conditions in the Atlantic coastal regions. In this respect it is important to note that extensive surveys of woodland in Belgium, revealed only minor plots and limited coverage with larch, and also only very few cases of *P. ramorum* infections (K. Heungens, Instituut voor Landbouw en Visserij Onderzoek, Merelbeke, Belgium, pers. com.). The situation in the Netherlands seems very similar, although according to the Dutch Forestry Organization (Staatsbosbeheer) there is a very limited amount of data available regarding the spread of *P. ramorum*, apart from the initial host species *Rhododendron* and *Viburnum* and a few infected loci found on red oak (*Quercus rubra*) and beech (*Fagus sylvatica*)

(Letter from Foundation "Samen voor NP Sallandse Heuvelrug" to the author, 22 January 2019) (See also Figure. 3 C, D).

In the following paragraphs, mixed models will be presented for modelling the spread of infectious pathogens in woodland areas consisting of a mixture of fast growing tree plots (e.g. larch) and in extremely slow-growing moss and lichen-covered 'pristine' plots. Also the seasonal variation of pathogen dispersal via asexual sporangia, chlamy dospores and sexual oospores is discussed (5).

Mixed Spatial Models for The Spread of Infectious Pathogens

For the clarity of argument, we present two limiting cases of heterogeneous spatial domains: in the A-case spatial domains with high (H) resistance to the spread of *Phytophthora* sp. represent the dominant vegetation type (e.g. old, moss- and lichen-covered woodland) and corridors of low (L) resistance are filling the remaining area (new forests or non-covered woodland or non-wooded area) (Figure. 5A). In the B-case, the dominance-type is reversed: the low resistance domains dominate over the high resistance domains. This approach is in line with studies where the propagation of a disturbance (e.g. an infection) is modeled in heterogeneous populations with high and low host densities [21], also designated as 'patchy spread' reaction-diffusion models [22]. The models presented here belong to the so-called mixed models, resulting from the combination of (isotropic, radial) diffusion dynamics [23] and (linearized) adsorption dynamics [24], as previously used in reaction-diffusion modeling [25].
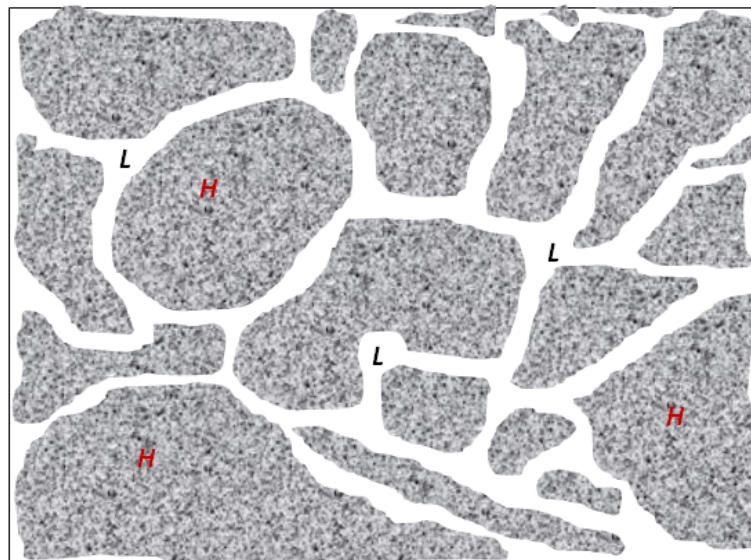

Figure 5A. Graphical representation of mixed spatial model, containing high resistance (H) and low-resistance (L) domains.

An important outcome of a deterministic model for patchy spread is that the propagation becomes damped when there is a strong Allee effect [26], e.g. at a low population density.

In the homogenous, isotropic situation, Diekmann and Heesterbeek (2000) postulated a linear diffusion equation, formulated as

$$u_t = D\Delta u + \kappa u \quad [1]$$

with u_t the partial derivative of u with respect to time, D the species-dependent diffusion constant and Δu the second partial derivatives with respect to two coordinate directions. It was shown that an asymptotic speed of propagation c_0 may be derived, which equals the minimal speed for which plane travelling wave solutions exist [23]. The following solution of the linear diffusion equation describes the result of a localized disturbance (at x = 0 and t = 0):

$$u(t,x) = \frac{1}{4\pi Dt} e^{-\frac{|x|^2}{4Dt} + \kappa t} \quad [2]$$

The asymptotic speed of propagation inferred from this solution [2] is expressed as

$$c_0 = 2\sqrt{D\kappa} \quad [3]$$

In a system of two competitive prey species (or a system with so-called obligate mutualism) and a consuming predator, a pattern of 'patchy spread' may arise [22]. Whereas in earlier studies, heterogeneity because of variation in the invasion speed in different space directions was attributed to an environmental heterogeneity [21], according to Morozov et al. [22] also an internal mechanism of system dynamics may explain the observed spatial heterogeneity.

In their model, three coupled reaction-diffusion equations are used:

$$\frac{\partial u_1}{\partial t} = \Delta u_1 + u_1(1 - u_1 - \chi_{12}u_2) - \frac{u_1 v}{1 + \gamma u_1} \quad [4]$$

$$\frac{\partial u_2}{\partial t} = \varepsilon_1 \Delta u_2 + r u_2(1 - u_2 - \chi_{21}u_1) \quad [5]$$

$$\frac{\partial v}{\partial t} = \varepsilon_2 \Delta v + g \frac{u_1 v}{1 + \gamma u_1} - \delta v \quad [6]$$

Where u_1, u_2, and v are the densities of competing preys (u_1,u_2) and predator (v) (in our example to be replaced by the host species and the pathogenic fungus), r is the maximal dimensionless growth of the inedible/unaffected prey/tree species, and χ_{12}, χ_{21} describe the intraspecific competition (between the tree species) (after [22]). Moreover, the parameters γ, *g* represent the feeding of the predator/parasite and δ is the mortality rate of the predator. The introduction of a mortality rate (δ)is an important controlling parameter, in order to be able to control or mitigate the spread of infectious species or pathogens, as demonstrated by Fagan et al. [27]. Morozov et al. [22] demonstrated that a transition to patchy spread from initial regimes with smooth fronts (or so-called traveling wave population fronts) may take place via the propagation of concentric rings.

The fact that in many mixed woodlands, the patchy pattern is man-made and the heterogeneity is not the result of a natural dispersal phenomenon, is not problematic: it simply means that the initial conditions for the model are already corresponding to a patchy pattern. Hence, no transition from a homogenous and isotropic regime to this patchy pattern is necessary. For modelling the further spread of so-called propagules (in our example the spread of the *Phytophthora* fungus) Hengeveld [28] introduced the notion of the 'dispersion probability field'. According to Hengeveld, with increasing distance from the source area, the proportion of propagules dropping always shows an exponential distribution. The exponential decrease can be formulated as:

$$\log(G(x,t) = -[(x - v)/a]^b \quad [7]$$

with G(x,t) representing the proportion of propagules having travelled a distance x at time t, v represents the degree to which the propagules moved in a certain direction, and a the mean distance covered [28]. The parameters a and v are time-dependent and, in absence of drift, e.g. because there is no preferential wind direction, the time-averaged parameter v equals zero. In the A-case presented above, an adaptation of this formula following the linear distance of the corridor-like domains is possible (Figure. 5 B). If the proportion of propagules that are dropped outside the corridor with low resistance are equalized to zero, and x representing the linear distance in the length direction of the corridor domain, then v may be equally put to zero.
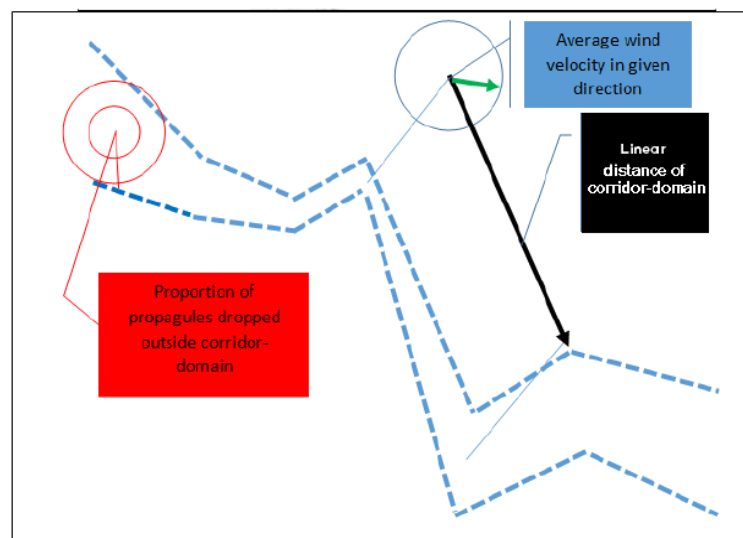

Figure 5B. Simplified linear model parameters for modeling the spread in the A-case mixed spatial model.

Furthermore, an important result of the work of Morozow et al [22]. is that the spatial distribution is important to understand the coexistence of competitive (predator/parasitic) species in the long term. In other words, once a patchy pattern is formed, the non-homogeneity of spatial distribution continues to influence the spatial pattern of dispersal.

Mixed Temporal Models for The Spread of Infectious Pathogens

The epidemiology of *P. ramorum* infectivity, however, shows an augmented complexity, because of the combination of alternative routes for dispersal via airborne asexual sporangia, via long-lived chlamydospores that can survive in the soil for very long periods [29], and via the occasional formation of sexual oospores (see also Figure 2). Adaptations of the model is required to account for the cumulative effect of short term, quick dispersal and long-term survival.

In the spatial approach, the optimal spread of a predator or parasitic species - because of Allee effect [26] - depends on a sustained distribution of a population of prey or (infection) susceptible species. In the temporal approach, however, the survival fitness of a predator/parasite depends on all kinds of periodic fluctuations between predator or parasite (abundance) versus the prey or susceptible species (e.g. timing of reproduction, hatching, molting, etc.). This temporal pattern of survival fitness for instance is well documented in the family of periodical cicadas (Magicicadas). These insects have an exceptionally long reproduction cycle consisting of a prime number of year cycles. An extra-ordinary example of a fungal parasite 'hijacking' the 17-year reproduction cycle of *Magicicada septemdecim* recently became unraveled [30].

According to Murray [1], in a so-called three species model, travelling epizootic wavefronts can be introduced and their speed of propagation estimated according to a system of coupled differential equations (in a primarily homogeneous spatial domain). Herein, time and distance between recurring outbreaks are obtained from the model using estimates obtained from field data. Murray [31] uses the three species approach to model the example of the progress of a rabies epidemic in foxes, classified into susceptible foxes (S), infected, but non-infectious foxes (I) and rabid foxes (R). After transforming the S-I-R variables into the non-dimensional quantities s, q, r (depending on distance x and time t, and obtained by dividing S, resp. I, R, by the environmental carrying capacity, K), the model may be solved resulting in the following type of solutions:

$$s(x,t) = s_0 + A\cos\left[\omega\left(t + x/v\right) + \psi\right]\exp\left[-\lambda\left(t + x/v\right)\right] \quad [8]$$

which is the product of a periodic and exponential function of time and distance (from the source of the epidemic) and the so-called steady state solution (s_0, q_0, r_0) [31]. However, in this model the variables S,I, R are all interrelated and belong to a single biological species (*Vulpes vulpes*). It may not be surprising that when periodical functions are used, which represent patterns oscillating in time and space, a proper choice of the timing of the peak values results in optimal synchronization of the different patterns.

In case of the propagation of the *Phythophthora* pathogen, not only the susceptible species belong to various, different taxa, with different degrees of susceptibility and much more complicated patterns of interactivity between these tree species (with the sole limitation of the maximum carrying capacity for all tree species of an area), but also, the infectivity of a host tree may differ for the different asexual and sexual reproductive structures of *P. ramorum* (Fig. 2). In general, the overall probability for interaction depends on the 'time window' for infectivity and sporulation between host and parasite. However, we may gain some benefit from the analogous case of the reproduction cycle of periodical cicadas and their fungal parasites (see above).

From this analogy and the above solution [8] of Murray's equation, we may infer that the cyclic variation of host infectivity (Inf) and fungal parasite sporulation (Spo) are related to the following expressions, respectively:

$$Infect(x,t) \approx A(\cos\omega t + \psi) \quad [9]$$

$$Spo(x,t) \approx B(\cos\phi(t + x/v) + \psi) \quad [10]$$

In these expressions the amplitudes A, B are directly proportional to the physical conditions (temperature, wind speed, moisture, etc.), whereas the cyclic, biological interactivity in time depends on the periodicity factors ω, φ (with respective periods $2\pi/\omega$ and $2\pi/\varphi$) and a non-periodic infectivity factor ψ (for asexual and sexual reproduction types of *P. ramorum*). Hence, in this rather simplified model, the interactivity between host and parasite may be modelled in terms of the physical factors A,B and the biological periodicity factors ω, φ and some non-periodic factors. However, the applicability and the fine-tuning of this model depends on the availability of concrete numerical data to define these parameters in terms of empirical data from the field.

Concluding Remarks

It is currently well-known that air pollution has a detrimental effect on the growth of lichens, resulting in the absence of lichen coverage in woodlands with poor air quality [7]. At the outset of this paper, we formulated the main research question, namely "Can Lichens form a barrier for Fungi marching on?" and, more specifically, are they capable of "slowing down the spread of Sudden Oak Death pathogen *Phythophthora ramorum*?". This research question so far has raised a number of subsequent questions, which however so-far haven't been answered yet. However, we think that these sub-questions may be

instrumental to find answers to this complex problem in the future, which could be listed as an example of the global spread of fungal diseases, which is an important, if not primordial, global challenge following climate warming of the planet. It is also known that lichens contain particular chemical substances, some of which have been shown to possess distinct anti-fungal activities.

On the other hand, the occurrence of *P. ramorum* infection in adjacent areas in the same regions, where woodland areas harboring slow-growing lichens also occur, may seem contradictory to our main research question. However, fast growing trees in production forests outrun the growth rate of these lichens, so that lichens aren't capable of covering these trees in the short term.

In order to find answers to these questions, we pointed out a number of methodological difficulties. First, we definitely need more experimental information from the laboratory on chemical interactions between lichens and fungi. Discovering the presence of the putative inhibitors of important biochemical pathways is not sufficient in itself, therefore also the effectivity in an outdoor environment should be tested. Secondly, the interaction between lichens and fungi should be further examined in absence of large-scale interventions in these forests, which of course, could become problematic in cases of acute risks for the forestry harvesting. Therefore, we suggest non-disruptive ecological examination of lichen-covered and uncovered forests in a suitable, unpolluted geographic region.

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