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A Framework for Optimizing Phytosanitary Thresholds in Seed Systems

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ABSTRACT

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Seedborne pathogens and pests limit production in many agricultural systems. Quarantine programs help reduce the introduction of exotic pathogens into a country, but few regulations directly apply to reducing the re-introduction and spread of endemic pathogens. Use of phytosanitary thresholds helps limit the movement of pathogen inoculum through seed, but the costs associated with rejected seed lots can be prohibitive for voluntary implementation of phytosanitary thresholds. In this paper, we outline a framework to optimize thresholds for seedborne pathogens, balancing the cost of rejected seed lots and benefit of reduced inoculum levels. The method requires relatively small amounts of data, and the accuracy and robustness of the analysis improves over time as data accumulate from seed testing. We demonstrate the method first and illustrate it with a case study of seedborne oospores of *Peronospora effusa*, the causal agent of spinach downy mildew. A seed lot threshold of 0.23 oospores per seed could reduce the overall number of oospores entering the production system by 90% while removing 8% of seed lots destined for distribution. Alternative mitigation strategies may result in lower economic losses to seed producers, but have uncertain efficacy. Future challenges and prospects for implementing the approach are discussed.

The use of clean seed is an important component of disease and pest control in agriculture. Clean seed, sometimes referred to as ‘quality declared seed’ and ‘disease-free seed’, are seed lots that are certified to have pathogen incidence or severity below established thresholds (Thomas-Sharma et al. 2016). Use of clean seed reduces the primary inoculum entering a field and contributes to lowering the overall epidemic severity of both mono- and polycyclic diseases. Programs at state and federal levels help prevent introductions of exotic pathogens into the US, but strategies for slowing saturation by endemic pathogens are less commonly considered (Xing et al. 2017). Re-introductions of pathogen species pose an important risk of establishing novel genotypes or cryptic species that are difficult to distinguish from endemic pathogens (Atallah et al. 2010; Short and Gurung et al. 2015b). Managing these re-introduction events is critical to the long-term sustainability of US agriculture.

Global trade has increased the movement of seeds and other plant material between countries, increasing the risk of movement of plant pathogens (Epanchin-Niell and Hastings 2010). Exotic pathogen introductions can have catastrophic effects on both natural and agricultural ecosystems, resulting in large economic losses from lost ecosystem services and reduced yield (Atallah et al. 2010; Cunniffe et al. 2016; Epanchin-Niell 2017; Short and Gurung 2015a). Introduction of exotic virulent strains of endemic pathogens can also severely disrupt agriculture (Atallah et al. 2010; Short and Gurung et al. 2015b), and are difficult to regulate without a clear way to distinguish between the endemic and exotic virulent types. This is further complicated by the uncertainties of taxonomy, which frequently re-assigns deviating groups of isolates to novel species (Inderbitzin and Subbarao, 2014). Mis-identification of cryptic species as endemic pathogens allows for accidental introduction into new regions. Once

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a pathogen has established itself and is widely dispersed in a new region, eradication efforts are rarely successful (Epanchin-Niell and Hastings 2010). Endemic pathogens persist in the environment through several different means. Many plant pathogens produce durable survival structures that can persist in soil or plant debris for years, and other pathogens have wide host ranges, with multiple host species that can act as green bridges between different regions.

Phytosanitary thresholds are one way to mitigate the introduction of new exotic strains and to reduce the overall amount of pathogen inoculum moving into a region, by limiting the amount of allowable pathogen inoculum in or on affected plant parts (Gabrielson 1988; Kuan 1988; Russell 1988; Schaad 1988; Stace-Smith and Hamilton 1988; Roberts 1999). While exclusionary quarantines can be seen as an extreme version of a phytosanitary threshold, many seed systems employ non-zero phytosanitary thresholds, where a small amount of pathogen is allowed in infested seed lots. Non-zero phytosanitary thresholds are most frequently implemented in seed systems, where seeds can be externally infested or internally colonized by pathogen propagules. After germination and seedling emergence, seed-borne inoculum can contribute to the overall epidemic in a region. Reducing this primary seedborne inoculum is critical in many pathosystems for regional disease control (Wu and Subbarao 2014). Most phytosanitary thresholds take the form of allowable limits of pathogen density or incidence, and are determined by a standardized protocol.

While setting a threshold of zero detectable pathogen density or incidence would be in some ways desirable, it is often impractical for endemic pathogens. At the same time, having no limit on endemic pathogens can result in important economic losses to farmers in the short run, and increased breakdown of resistance genes in the long run. Programs for the development of quality-declared seed with lower pathogen levels can make important contributions to reducing regional disease levels and increasing the useful life of resistance genes. Such programs would benefit from strategies to develop phytosanitary thresholds for endemic pathogens that balance the need for adequate quantities of reasonably priced seed, with the need for reduced pathogen levels.

Here we outline a framework that offers guidance for developing suitable phytosanitary thresholds using available data, and helps reduce multiple re-introductions of endemic pathogens on seeds. We offer an outline for estimating the effects of thresholds at individual field and regional scales, and we describe the costs and benefits of establishing these thresholds. Thresholds derived from empirical analyses such as those used here need to be recalibrated as changes in agricultural and climatic conditions (such as host density and environment) occur and as (if) new data become available from additional seed testing.

Our objectives are to: (1) develop a general framework for establishing phytosanitary thresholds, given the common limitations on data availability, for scenarios where non-zero thresholds are useful; (2) explore the integration of landscape epidemiology for establishment of phytosanitary thresholds; and (3) apply this framework to spinach downy mildew, an important endemic pathogen of spinach in the United States and globally, that is repeatedly re-introduced via infested seeds.

WHEN ARE PHYTOSANITARY THRESHOLDS USEFUL?

Phytosanitary thresholds limit the amount of pathogen inoculum allowed on infested plant parts. Phytosanitary thresholds are most useful in systems where an endemic pathogen is repeatedly re-introduced on seeds or other incoming plant parts (Fig. 1). These re-introduction events can introduce new virulent genotypes or mating types, allowing the pathogen to adapt more readily to control strategies such as resistant varieties or

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fungicides. Developing a universally accepted phytosanitary threshold may require years of laboratory and in-field experiments that demonstrate the link between pathogen levels and economic damage from a disease (Kuan 1988; Wu and Subbarao 2014), and many diseases are difficult to control through phytosanitary thresholds alone (Grogan 1980). Some questions can help producers decide if a phytosanitary threshold would be useful for their pathosystem (Fig. 1):

Is the pathogen exotic? While non-zero phytosanitary thresholds help reduce the amount of inoculum introduced into a region, they do not fully remove all inoculum. In many cases, the most economic and effective control for exotic or highly virulent pathogens is with an exclusion quarantine (Epanchin-Niell 2017). Using a non-zero phytosanitary threshold for an exotic pathogen is counterproductive, and increases the risk of future outbreaks and increased economic losses. These threats are compounded in systems involving internationally quarantined agents, where the risk of regional colonization is not simply yield loss but also a reduction in trade (Marshall et al. 2003; Stansbury et al. 2002; Brennan et al. 1992).

Does the pathogen persist in the environment? Pathogens that are capable of forming durable survival structures may persist in soils for several growing seasons, allowing for the accumulation of inoculum over time (Atallah et al. 2012). Phytosanitary thresholds that allow pathogen inoculum from infested seed lots into fields can create enduring problems for production (Short et al. 2015). Phytosanitary thresholds for pathogens that form durable structures should account for agronomic practices, such as rotation strategies and host susceptibility.

Does the pathogen spread rapidly, even at low densities? Developing an effective long-term solution for easily-spread pathogens can also be hindered by the effects of secondary inoculum contributing to the regional epidemic (Fig. 2). Early applications of seed thresholds may appear to fail if the effects of secondary inoculum are large, allowing disease to develop in plants grown from clean seeds (Grogan 1980). Implementing the threshold consistently over a wide area along with other control efforts to reduce secondary inoculum can eventually cause disease incidence to decline. However, this effect depends on whether the combined mitigation tactics can reduce the basic reproductive number (R_0) to below one. If the overall reproductive number remains above one, outbreaks will continue to occur in the region. One example of a successfully implemented disease threshold is in lettuce mosaic disease in coastal California, where a combination of clean seed, vector management, removal of alternative weed hosts, and a host-free period in the winter effectively controls disease outbreaks (Grogan 1980). These combined efforts have reduced the severity of lettuce mosaic disease in coastal regions where it once resulted in severe yield loss.

Does the incoming inoculum contribute to pathogen diversity? Many plant pathogens have spatially structured populations (Goss 2015; Grünwald and Goss 2011). Movement of infested seed may introduce novel genotypes, increasing local diversity and degrading efficacy of control strategies such as host resistance and pesticides. The genetic structure of many pathogens is poorly understood, making it difficult to assess the extent of increasing genotypic diversity. The presence of a reproductive stage on infested seed may pose extreme concern for producers because of the risk of generation of novel genotypes through recombination and selection (McDonald and Linde 2002).

COSTS AND BENEFITS OF PHYTOSANITARY THRESHOLDS

An ideal phytosanitary threshold would maximize the total amount of inoculum removed, minimizing the number of seed lots removed by the threshold, while maintaining economic disease control. The tradeoff

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between lost seed lots and contaminated seeds removed is difficult to balance because phytosanitary thresholds can vary in their efficacy depending on the region. For example, lettuce mosaic virus can be controlled in the Netherlands by using seed certified to have 0 infested seeds in 2000, while in California the necessary threshold is 0 in 30000 (Kuan 1988). Differences in climate, agronomic production density, and vector populations create greater risk from a small number of infested seeds in California.

Costs: Establishment of effective phytosanitary thresholds requires thorough field and laboratory work. While some thresholds are developed over the course of several years and based on numerous studies, more frequently they are arbitrarily determined, or based on limited field observations (Kuan 1988). One problem in establishing thresholds is that the terms of international trade, ratified by the World Trade Organization (and previously by the General Agreements on Tariffs and Trade – GATT), specifically prohibit participating countries from erecting arbitrary barriers to trade (Henson and Loader 2001). Phytosanitary concerns are legitimate reasons to impose trade import bans, or actions to reduce risk by the exporting country, but if thresholds on seed contamination are set arbitrarily they are susceptible to challenge.

Seedborne pathogens heavily affect agriculture in the developing world, where they are especially important for vegetatively propagated crops, which are the staple carbohydrate for many subsistence farmers (Sperling, Ortiz, and Thiele 2013; Xing et al. 2017; Hernandez Nopsa et al. 2017; Thomas-Sharma et al. 2016). While there is a pressing need to reduce incoming inoculum on infested seed lots, strict thresholds may be too costly to implement. A phytosanitary threshold that aims to remove all inoculum may be unattainable for these settings, resulting in no quality-declared seed at all. A non-zero threshold may provide many benefits by reducing levels of infection while still being economically viable for smallholder farmers.

Evidence-based or precautionary, all phytosanitary policies have costs. In addition to short-term costs, such as the cost of removed seed lots, there are potential long-term costs; e.g., potential retaliatory quarantines, reduced seed supply, and lost planting opportunities. Broadly, these wider long-term costs range in the nature of their impacts from the purely private to the public, and all are subject to compounding effects over time as pathogen prevalence increases in a region, as a consequence of continued import and secondary increase. In a similar way, the potential benefits of a phytosanitary threshold range from public to private and may be considerable when summed over large areas and long timespans.

Benefits: The benefits associated with phytosanitary thresholds depend on both the spatial and the temporal scale considered. At a small temporal and spatial scale, phytosanitary thresholds help to reduce the amount of inoculum introduced into a single field, reducing the risk of economic losses due to disease. Over time, this reduction in inoculum can help reduce regional disease pressure by reducing genotypic diversity of the pathogen. Pathogen introduction events can directly introduce new virulent strains from other regions, or can increase the population diversity by introducing new mating types or sexual structures. Minimizing the genotypic diversity of the pathogen population stabilizes the efficacy of control strategies. The risk of development of pesticide resistance and host resistance-breaking strains increases with large increases in pathogen populations (McDonald and Linde 2002; Stukenbrock and McDonald 2008). Some of these effects are only seen when considering an entire landscape of susceptible hosts.

MEASUREMENTS OF INOCULUM ON SEED

Being able to quantify seed lots for

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pathogen presence accurately and consistently is important for the development of effective phytosanitary thresholds. The sampling theory associated with this process is well developed (Geng et al. 1983; Russell 1988), and calculating a necessary sample size given accepted error rates is fairly straightforward (Geng et al. 1983; Madden et al., 2007). However, using these measurements to construct an effective phytosanitary threshold can be challenging.

Seed assays vary based on the type of pathogen being detected (fungal, bacterial, viral) and the sensitivity required of the assay. Some assays determine inoculum density, a measure of how much inoculum is present per unit of seed (Fig. 3). The density of bacterial seed pathogens is frequently quantified as colony forming units (CFUs) per gram of seed (Schaad 1988). Other assays directly detect the infestation status of individual seeds, and are frequently expressed as the proportion of seeds affected, a measure of disease incidence (Pryor et al. 1994). Each of the different types of assay has advantages and disadvantages, given different pathosystems. It might be effectively impossible to conduct visual assays of individual seeds when sample sizes are large, making incidence assays unusable. For pathogens with high reproduction rates, assays based on pathogen inoculum density may not be more informative or useful than incidence assays, since reproduction by the pathogen, once introduced into the system will quickly minimize variation in primary inoculum on seeds. Of the three data types, inoculum density may be the most difficult to use for routinely applying phytosanitary thresholds. Density assays rely on accurate identification of pathogen propagules, which may be difficult due to excess contamination, or the presence of phenotypically similar pathogenic and non-pathogenic strains.

Both density and incidence type assays can be expressed as presence/absence assays, where, in the case of density measures, the population density scale is partitioned into two classes, zero and all population densities greater than zero. Any assay expressing results at the seed scale can also be used to generate incidence (i.e. presence/absence) data at the scale of the seed lot. Such seed lot scale results can be useful in cases where strict control of pathogen inoculum levels are necessary to maintain effective disease management.

Irrespective of the type of data collected or the pathosystem that is the target of the screening activity, an operational decision rule is required in order to sort seed lots into “accept”, “reject”, and perhaps “re-test” categories. The decision rule will always be applied based on a sample drawn from the seed lots, and the long-term statistical performance of the decision will depend on how representative the sample is. Researchers should re-evaluate the initial decision rule as new data accumulates from continued testing.

The capacity to use thresholds effectively depends on the relationship between the intensity of infection and the distribution of infection in seed lots. Ideally, a high proportion of pathogen inoculum would be concentrated in a relatively small number of seed lots, so that these can be removed at low cost, while the majority of lots have a low level of contamination. If the cumulative proportion of seed lots is plotted against the level of infection, the resulting relationship should be convex for the threshold approach to have a high chance of success. In this case, the highly contaminated lots can be excluded by setting a relatively high threshold, allowing most seed lots to pass. In cases where inoculum is evenly distributed across seed lots or most seed lots have heavy infestations, the cumulative distribution of seed lots against contamination intensity is concave. In that case, a relatively high proportion of seed lots is excluded even at a low threshold level.

LANDSCAPE EPIDEMIOLOGY OF PHYTOSANITARY THRESHOLDS

Landscape epidemiology in plant pathology is typically concerned with the short- or long-

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distance transport of secondary inoculum, allowing neighboring regions of hosts to become infected. Pathogen propagules associated with seeds often serves as primary inoculum, directly affecting the initial disease status of a host. Estimates of the number of infection events that might occur under different scenarios can inform choices among different management options. Considering the consequences from seed phytosanitary thresholds at a landscape (or regional) level is important because the aggregate costs and benefits at such scales are likely to be the primary drivers of decisions to introduce seed inspection rules or not.

There is frequently not enough field or laboratory data to state definitively what the appropriate threshold should be; this makes setting evidenced-based policy problematic (Kuan 1988). Data might simply be too difficult or costly to collect. Separating the effect of seed contamination on disease levels from other confounding variables may be experimentally challenging. Secondary inoculum or differences in regional environmental effects or host populations may cloud the effects of seedborne inoculum on the regional epidemic progression (Grogan 1980; Kuan 1988). With the difficulties in establishing evidence-based policy, the temptation is to use a general precautionary approach, but this can be overly restrictive of trade.

The method of Fermi approximation (Edge and Dirks 1983) can be used to obtain probable order-of-magnitude estimates of the level of infection that likely would arise from a given level of seed contamination. Although the estimates are imprecise, these methods are useful when there is relatively little direct information about a subject. The estimation of infection levels may be distorted if there are multiple sources of primary inoculum (e.g. overwintering infected hosts, durable survival structures in soil or field debris, neighboring infected fields, etc.).

If pathogen population density per seed is measured, a Fermi approximation of the total potential infections (*I*) across a landscape from infested seed lots, can be obtained, starting with estimates of the inoculum load per seed obtained from individual samples:

$$x_t = \frac{x_i * (\frac{t}{s})}{l} \text{ (equation 1)}$$

where x_t is the estimated total number of propagules per seed in the seed lot, x_i is the number of observed propagules reported from the subsample, *t* is the total amount of sample, *s* is the amount of subsample analyzed, and *l* is the total number of seeds tested in the sample. Equation 1 accounts for differences in the sampling proportion each sample represents across seed lots of different sizes. With data for the average inoculum load per seed, and the proportion of infected seed lots obtained from sample data, the Fermi approximation for the number of infections arising from infected seed (*I*) is given by:

$$I = \bar{x}_t * S_i * R_S * R_G * R_i * A_T \text{ (equation 2a)}$$

where $\bar{x}_t$ is the mean number of propagules per seed across the infested seed lots, S_i is the proportion of seed lots that are infested, R_S is the seeding rate per unit area, R_G is the germination rate, R_i is the infection rate (infections per propagule), and A_T is the total area of susceptible plants. If we assume that the seeding rate, germination rate, infection rate, and acreage of susceptible crop are constant between seed lots that are heavily- and lightly-infested, then we can simplify the expression to represent a proportional risk factor (θ), or

$$\theta = \bar{x}_t * S_i \text{ (equation 2b)}$$

If we assume that management strategies like seed lot removal affect the total number of pathogen propagules but do not affect the pathogenity of the remaining propagules, we can use θ to estimate the relative effect of different management strategies by comparing the untreated proportional risk factor (θ_u) and the treated proportional risk factor (θ_t) to determine the proportional difference in effect

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of different management strategies.

There are a number of open questions beyond estimating the number of total infections occurring over a landscape. While pathogen density assessments determine an average inoculum load across a set of tested seeds, inoculum is frequently unevenly distributed (Danielsen et al. 2004), with some seeds being heavily infested and others being relatively clean. Understanding how this uneven inoculum distribution may be used in predicting the spatio-temporal progress and aggregation of the disease in affected fields is an open question (Fig. 3). Many pathosystems, including some seed systems (Roberts 1999), have epidemiological thresholds, where an initial level of infection from seeds are low enough to keep epidemics from spreading beyond the area of initial infection (Gilligan and van den Bosch 2008). Understanding the factors that lead to locally-contained versus widely-spreading epidemics could help to set more economically reasonable phytosanitary thresholds.

CASE STUDY: SPINACH DOWNY MILDEW

Downy mildew caused by the obligate oomycete pathogen *Peronospora effusa,* is the most important disease of spinach (Correll et al. 2011). Disease symptoms are characterized by chlorotic lesions on leaves, and severe symptoms can lead to post-harvest decay, resulting in reduced quality and shelf life of fresh market types (McKay et al. 1992). Most spinach production in the United States occurs in California and Arizona, primarily in the Salinas, Imperial, and Yuma Valleys (Koike et al. 2011). *Peronospora effusa* is endemic in California with annual epidemics that intensify as production increases during each season (Choudhury et al. 2016).

In the fresh market sector, organic spinach represents about one third of total production in the US (USDA NASS 2015). Growers rely heavily on resistant varieties to prevent disease, especially for organic spinach, which cannot be sprayed with synthetic pesticides. New, virulent pathogen races have emerged at an exponential rate (Correll et al. 2011) over the last two decades. This rapid emergence of new races has disrupted spinach production by forcing spinach breeders to focus on resistance traits, and to develop new resistant varieties (Correll et al. 2011). The escalation in the appearance of pathogen races has occurred concurrently with intensification of production methods, such as the use of wider planting beds, increased seeding rates, and use of overhead irrigation (Correll et al. 2011; Koike et al. 2011).

The multiple simultaneous changes within spinach production over the last 20 years make it difficult to partition which factors most significantly influence the emergence of new pathogen races. Irrespective of its causes, an increasing diversity in *P. effusa* has led to increased disease outbreaks, allowing the pathogen more rapidly to overcome resistance genes deployed in new varieties. While some oomycetes can undergo changes in population diversity through asexual reproduction alone (Lamour et al. 2012), evolutionary models suggest that pathogens capable of both asexual and sexual reproduction represent the highest risk for developing higher population diversity and overcoming resistance genes (McDonald and Linde 2002). A recent population study provides some evidence for sexual reproduction of *P. effusa* (Lyon et al. 2016), and identifies common genotypes in geographically diverse areas, suggesting either inter-regional dispersal or a common source of *P. effusa*.

The recent detection of viable oospores in commercial seed lots raises the concern that new races of spinach downy mildew might be arising from sexual reproduction (Kunjeti et al. 2016), but the extent to which this is occurring in a given geographic area is not clear. New races may already be present in the population and their presence simply revealed after the deployment of new resistant cultivars that provide high levels of selection against other genotypes (Correll et al. 2011). Reducing the

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number of oospore-infested seed lots entering the production system each year may benefit growers and seed producers by delaying or stopping the emergence of new pathogenic races.

Oospore infested commercial seed lots. Eighty-four untreated, organic spinach seed lots from multiple commercial seed producers were tested for the presence of oospores in a procedure similar to that described in Kunjeti et al. (2016) (Fig. 4, Supplementary Table 1). Briefly, 500 or 1000 seeds were placed into a 50-ml Falcon tube with 20 ml of sterile de-ionized water. Tubes were fixed onto a Fisher pulsing vortex mixer for 10 minutes at full power. The liquid was eluted through one layer of cheesecloth and centrifuged for 10 minutes at 4000 RPM in an Eppendorf 5430 centrifuge. The pellet was resuspended in 500µl of sterile de-ionized water and examined under a light microscope at 100× magnification for the presence of oospores. Oospores were identified as *P. effusa* by morphological characteristics based on previously described reports (Choi et al. 2007; Inaba et al. 1983; Kunjeti et al. 2016). The results are representative of the average number of oospores observed in three replicates of 75 µl of the resuspended pellet. When possible, the diameter of ten oospores per infected seed lot were measured for comparison with previously reported measurements of oospore dimensions (Choi et al. 2007; Inaba et al. 1983; Kunjeti et al. 2016).

Using equation 1 we can calculate the density of oospores per seed in the samples from these studies (Fig. 4). For example, seed lot number 29 from Kunjeti et al. (2016) reported 180 average oospores in one-75 µl measurement from a 500 µl resuspended pellet from a sample of 1000 seeds. So the total estimated number of oospores per seed in the seed lot (x_t) is equal to ((180 oospores * (500 µl / 75 µl)) / 1000 seeds), or 1.2 oospores/seed. This is an estimate of the number of oospores per seed, and may differ from the true value due to sampling error, and effectiveness of the oospore-removal process. This method was used to estimate the number of oospores per seed from two previously published studies (Inaba et al. 1983; Kunjeti et al. 2016) and from the new measurements we report here (Supplemental Table 1).

Estimating an optimum phytosanitary threshold for spinach downy mildew. An efficient economic phytosanitary threshold needs to balance the number of seed lots removed with the amount of inoculum removed. In general terms, if we assume that the total sales value of seed lots in a season is $$V$ and the total cost incurred from infections arising from seed in the absence of a phytosanitary threshold is $$C$, and that enacting a threshold results in the loss of a proportion, p_n, of seed lots from the system and a reduction, r_n, in the proportion of infections, then economic benefit occurs for all threshold values (n) such that $r_n C \geq p_n V$. Ignoring the identity of winners and losers for the moment, the optimal strategy would be to pick the value n, such that the two sides are equal. We can find the value of n by considering the ratio $\rho = V/C$. To balance the production costs saved due to reduced infection and seed value lost we need the ratio $f_n = p_n/r_n = 1/\rho$.

The lost value in rejected seed, V, represents a cost to the seed industry, while the costs of infection, C, represent losses to the production industry and it is not clear that balancing these two values will be either practical or politically desirable; in any case, depending on the economics of production for any given crop a feasible value for f_n may not be obtainable. The main point here is that the analysis provides a rationale for making objective decisions; a number of alternative, objective starting points for similar rational discussion can also be selected from the analysis. For example, taking Fig. 5A as the starting point, one could choose any one of three factors to consider as the most important for driving decision-making: a threshold value of oospores/seed, a proportion of seed lots to be

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retained¸ or a proportion of pathogen seed-borne inoculum to be removed.

Scenario (i) Regulatory Approach. In some cases, seed thresholds for pathogen contamination are set arbitrarily based on approximations or similar pathosystems. In the case of spinach downy mildew, selecting a threshold of 0.5 oospores per seed would result in approximately 15% of the potential oospore population on seed being accepted, and 94% of seed lots being passed for sale. This example is indicated by the vertical solid line rooted at 0.5 on the abscissa in Fig. 5A, and passing upward through the cumulative distribution curves for seed lots and oospore seed population density.

Scenario (ii) Benefit Seed Producers. Seed thresholds directly cost seed producers by increasing the number of rejected seed lots. Setting a loose threshold that reduces the number of rejected seed lots can help offset these costs while still reducing the amount of introduced inoculum. This example is indicated by the long-dashed lines (marked (ii)) in Fig. 5A, where a target of retention of 98% of seed lots is selected, resulting in acceptance of 55% of potential pathogen inoculum; the screening threshold is 1.2 oospores per seed.

Scenario (iii) Benefit Growers. Seed thresholds directly benefit growers by reducing the amount of inoculum on seeds. Selecting a stricter threshold reduces the amount of inoculum, but increases the number of rejected seed lots. A Fermi approximation (see above) can help determine a regional target for pathogen population reduction (Fig. 5A). Locate the value of the target on the y-axis and draw a horizontal line until it intersects with the pathogen population cumulative density function (CDF). Project vertically upward until the projection intersects with the seed lot CDF to reveal the fraction of seed lots that will be retained as a consequence, while projecting downward to intersect with the x-axis will indicate the operational phytosanitary threshold (oospores/seed) required to achieve the target. This example is indicated by the dotted black lines in Fig. 5A in which a target of 90% of pathogen inoculum is removed. This requires rejection of 8% of seed lots and is achieved with a screening threshold of 0.23 oospores/seed.

Scenario (iv) Maximize Proportional Difference. Calculate the difference between the CDFs for the pathogen inoculum and seed lots (Fig 5B). For a convex CDF for seed-borne inoculum and concave CDF for seed lots, the difference has a maximum value. Selecting this maximum results in an operational screening threshold that, on average, maintains the largest possible differential between proportional loss of seeds lots and removal of pathogen inoculum. Based on the available data for spinach seed, this corresponds to a threshold of 0.05 oospores per seed and results in the removal of 98% of the pathogen inoculum and 13% of the seed lots.

Operational thresholds depend on a concave trade-off between the proportion of seed lots rejected and the proportion of pathogen inoculum removed. The empirical trade-off in the case of the *P. effusa* oospore data used in this study is shown in Fig. 6. The key point, also indicated by the location of the maximum value in Fig 5B, is that the proportion of pathogen inoculum removed is much larger than the proportion of seed lots removed over the initial section of the relationship; large gains in pathogen exclusion for relatively small losses of seed must be possible.

Prioritizing Landscape Reduction in Infection. In Scenario (iii) given above, we indicated that priority could be given to reducing the burden of infection arising from infected seed when setting the phytosanitary threshold. We can estimate the total number of potential infections in the landscape using equation 2a. Total infections depends on the number of propagules in all seed lots and the number of seeds expected to be planted in the region. Based on the seed lots used in our study, 22% of the seed lots had observable oospores, and the mean number of oospores in infested seed lots was estimated as 0.45

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oospores per seed. Spinach has a germination rate of approximately 90% (Katzman et al. 2001), and is seeded at 8-9 million seeds per hectare (Koike et al. 2011), with approximately 7300 hectares of organic spinach in the United States (USDA NASS 2015), mostly concentrated in coastal California and the deserts of southern California and western Arizona.

Estimating the infection rate of *P. effusa* oospores is difficult, as there are no studies that have directly demonstrated oospore infection. However, a study in Japan (Inaba et al. 1983) measured both the number of oospores in seed lots and the number of infected seedlings that emerged from the infested seed lots. A linear regression of these two values (Fig. S1) suggests that there were approximately 1.5 infection events for every hundred oospores in a seed lot. Assuming a 1.5% infection rate based on these data, we can estimate that the total number of infections caused by oospores is approximately 8.8×10^{7} annually in USA spinach production. A phytosanitary threshold at 0.23 oospores per seed would reduce the number of infested seed lots to approximately 8% and the average number of oospores to 0.063 oospores per seed in infested seed lots. These reductions result in an estimated 4.5×10^{6} infections over the entire US. While direct oospore infection in *P. effusa* has not been observed, changing the infection rate used in equation 2a by orders of magnitude would still result in large numbers of potential infections in the United States. This suggests that while our estimate of a 1.5% infection rate may over-estimate the true infection rate, even large reductions in the infection rate would have significant effects over an entire landscape. For example, reducing the estimated infection rate from 1.5% to 0.0015% would still result in a potential approximate 4.5×10^{3} infections if no seed threshold was implemented.

Using equation 2b, we can estimate the proportional risk factor for both the untreated group of seed lots and the set of seed lots after using a phytosanitary threshold. We can use θ to estimate the relative effect of different management strategies by comparing the untreated proportional risk factor (θ_u) and the treated proportional risk factor (θ_t) to determine the proportional change effect of different management strategies. For example, if there are an average of 0.45 oospores per seed in 22% of all seed lots, and a 0.23 oospore per seed threshold reduced those levels to an average of 0.056 oospores per seed in 8% of seed lots, we could compare the θ_u and θ_t values as $\frac{0.45*0.22}{0.063*0.08}$ or 19.6, meaning that the untreated strategy has approximately 20× more risk of disease.

Cost of Mitigation for Oospore Infested Seed Lots. The worldwide movement of spinach seeds is extremely valuable, because most modern varieties are hybrids that can only be produced in a few regions of the world (Koike et al. 2011). Most commercial spinach production in the US is grown as baby-leaf clipped spinach, where the plants are grown at high density for approximately 30 days and then harvested. At a seeding density of approximately 8-9 million seeds per hectare (Koike et al. 2011), on approximately 7300 hectares of organic spinach in California and Arizona (USDA NASS 2015), and at an estimated price of $450 per million seeds (Tourte et al. 2015), the value of organic spinach seed sold annually is $32.4M. There are several mitigation tactics to reduce the proportional risk of oospore infection in organic seeds.

Total removal. The simplest and most costly tactic is to remove all infested seed lots from the market. This tactic has the advantage of guaranteed removal of oospore infection risk from those seeds. However, while the economic costs associated with total removal vary depending on the chosen threshold (Fig. 7), this tactic might be too costly overall for most commercial seed producers to consider. While infested seed lots may be discarded after a

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phytosanitary inspection to meet the objective of reducing the spread of pathogens, this might be a great economic burden on seed companies and on growers with reduced seed availability and crop production.

Seed Treatment. Several seed treatment methods have been developed, including physical treatments (e.g., steam, hot water, surface maceration), chemical controls (e.g., fungicides, bleach), and biological control (e.g., plant growth promoting microbes). These control tactics have different efficacies and impacts on seed germination. While biological control through the use of beneficial microbes is promising as a seed treatment option, the treatments frequently do not have the same efficacy or consistency as some other means of control (du Toit 2008; 2009). The cost of organic seed treatments is estimated to be approximately $0.10 per 1000 seeds.

Sell as Conventional. Conventional spinach is worth approximately 1/3 of the value of organic (USDA NASS 2015), therefore we estimate that the value of conventional seed is roughly $150 per one million seeds. Synthetic pesticides effectively manage downy mildew disease in conventional spinach. However, there are a few drawbacks to this tactic. Many organic varieties are selected solely for their downy mildew resistance profiles and may not have optimal horticultural properties, which may limit their use in the conventional market. In addition to this, while conventional spinach is currently successfully controlled using synthetic pesticides, exposing seed lots that are heavily infested with *P. effusa* oospores to synthetic fungicides may increase the risk of selection for fungicide resistance in *P. effusa* populations (McDonald and Linde 2002).

Costs. We estimate that given an optimal threshold of approximately 0.23 oospores per seed, complete removal would cost ~$2.7M (Fig. 7), conversion of organic seed to conventional seed would cost ~$1.8M, and seed treatment would cost ~$0.6M. While these numbers might suggest that seed treatment is the most cost-effective route for long-term disease control, the effects of different mitigation strategies could impede success, or even create worse situations. Organic seed treatments can have mixed or unreliable efficacy when compared with conventional synthetic pesticide treatments (du Toit et al. 2009, 2010; Cummings et al. 2008). Additionally, some organic seed treatments can reduce germination in spinach seeds below economically viable levels (du Toit and Hernandez-Perez 2005). Selling diseased organic seeds to conventional growers may concentrate heavily infested seed lots in one region or area. And as fields grown from seed lots with oospores are sprayed with conventional fungicides, the risk of developing fungicide resistance increases.

Ideal solutions may involve a mixed strategy combining multiple mitigation methods, depending on the efficacy of the different strategies. If we assume that removal of infested seed lots is the most efficacious way to reduce risk from oospore infection, and that organic seed treatment is a less costly solution compared with switching organic seeds to conventional, then an ideal strategy might involve complete removal of heavily infested seed lots and seed treatment of lightly infested seed lots. For example, complete removal of very heavily infested seed lots (e.g. – over 1.2 oospore per seed) and seed treatment of moderately infested seed lots (e.g. seeds with more than 0.23 and less than 1.2 oospore per seed) would cost ~$1.2M (Fig. 6), roughly half the cost of complete removal of all seed lots with >0.23 oospores per seed. Optimizing the different strategies depends largely on the efficacy of the mitigation strategies and the costs associated with each. Our current estimation of proportional risk (θ) does not directly factor in control strategy efficacy or long-term unintended consequences posed by treatments. Future work estimating these parameters would help guide mitigation practices.

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CONCLUSIONS, CHALLENGES, AND FUTURE PROSPECTS

Using a phytosanitary threshold to reduce the number of oospore-infested spinach seed lots has both costs and benefits. Complete removal of oospore-infested seed lots will greatly reduce the overall risk of the emergence of new pathogen races, but will cost the spinach industry a large portion of the usable seeds. The essential choice entailed in setting a phytosanitary threshold is how to balance seed producer and grower risks. Scenarios (ii) and (iii) in our analysis highlight the issue. Scenario (ii) illustrates a high acceptance rate of seed lots, and Scenario (iii) illustrates a low transmission of seed-borne inoculum. The seed producers' general goal for setting a threshold is that seed lots where the true level of infection is low have a low probability of being rejected. The seed buyers' goal is to ensure that seed lots with a high level of pathogen inoculum are rejected with a high probability. Our aim was to illustrate the general conditions under which these partially competing objectives can be satisfied and how empirical data from small-scale studies can be used as the basis for discussion. Madden et al. (2007, Section 11.3.1) provide a useful review of how producers' and buyers' risks can be used to design a formal sampling plan that will satisfy the competing requirements.

The overall pattern of pathogenic race emergence and the reduction in available resistant spinach germplasm (Correll et al. 2011) suggests that spinach downy mildew is becoming a more serious threat over time. If new varieties do not remain resistant to downy mildew long enough to recoup the cost of their development, organic spinach production will become unprofitable and unsustainable. The recent history of the industry suggests that new resistance types are frequently overcome after only a single field season, limiting their commercial lifespan. This suggests that without changes in the production system to account for managing pathogen population diversity and disease, US organic spinach production may disappear completely, as currently practiced. Reducing and delaying the emergence of new pathogenic races will require coordination, communication and cooperation between growers and seed producers.

The disease challenges facing spinach are a microcosm of agriculture in general. Recent changes in agronomic conditions, such as denser planting and a movement towards untreated organic products with lower tolerances for cosmetic damage, have occurred in many crops. These changes are frequently driven by new markets and reduced quantities of suitable irrigation water and arable land. In parallel with these recent agronomic changes, increasing globalization of agriculture and trade has increased the threat of widespread movement of virulent pathogens. Trade networks across the world connect previously isolated regions, increasing the threat of global plant pathogen pandemics (Xing et al., 2017).

Many seed systems exist as global networks of seed producers, breeders, and germplasm repositories. To make these systems resilient and adaptable to the introduction of new pathogens and pests (Garrett et al. 2017), phytosanitary thresholds should be set appropriately for each region in seed system networks. The level of phytosanitary thresholds could even be adjusted based on the position of a region in larger epidemic networks (Hernandez Nopsa et al., 2015). Phytosanitary thresholds could be especially valuable for less formal seed systems, where seed is traded between local growers and maintained by local non-governmental organizations. While overly strict phytosanitary thresholds may negatively impact these less formal systems, strategic phytosanitary thresholds could have a net benefit for the system as a whole.

Ultimately, reducing the amount of seedborne inoculum can help in both short- and long-term disease management. This paper provides a framework for optimizing

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phytosanitary thresholds for endemic pathogens that is relatively flexible depending on the data constraints of a new system. It is important to remember that these thresholds are regionally- and temporally-specific, and may need to be updated if agronomic or climatic conditions change in a region. Phytosanitary thresholds and clean seeds are a necessary management strategy for sustainable agricultural production in many systems.

ACKNOWLEDGMENTS

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Figures:

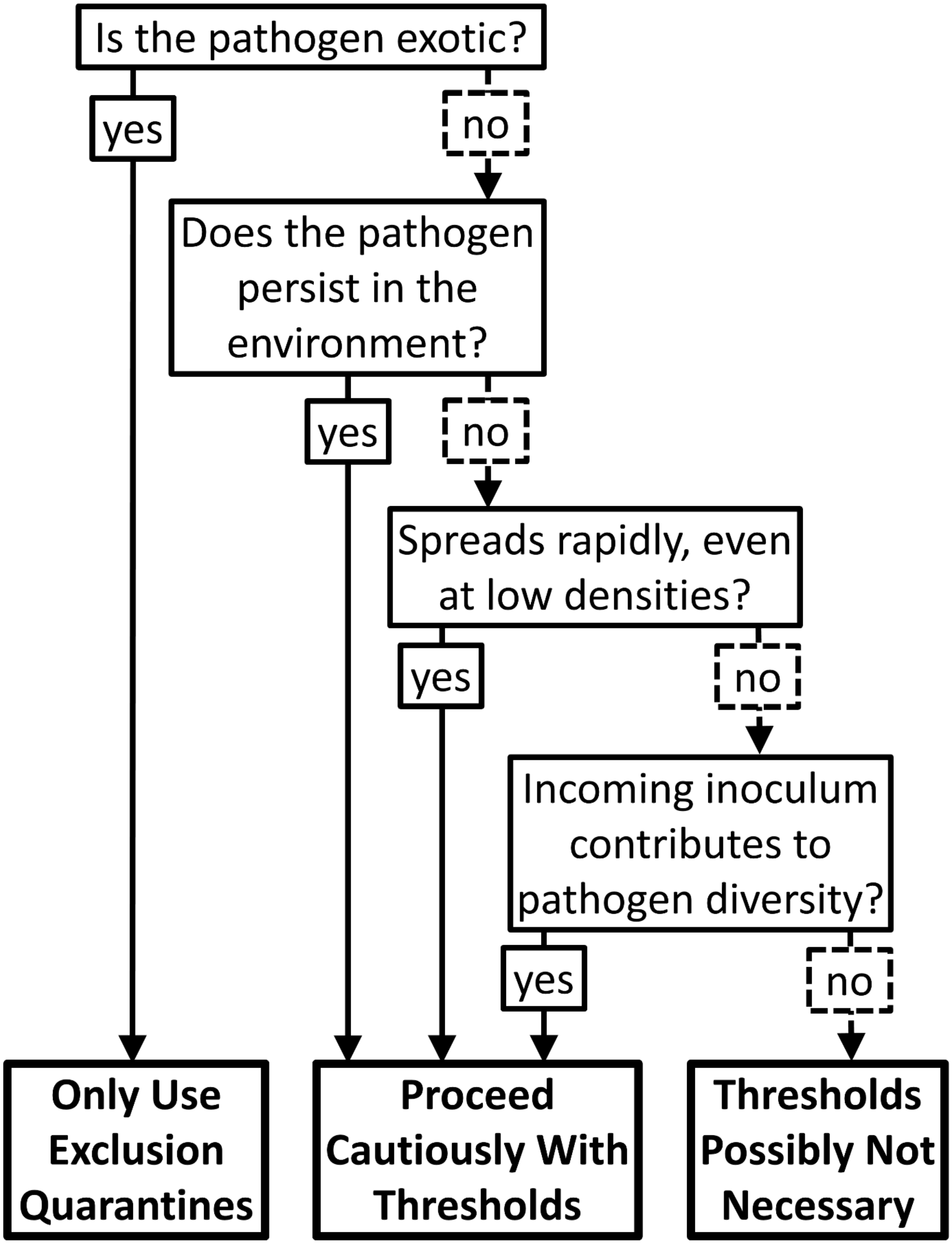

Figure 1. Decision tree for whether to implement phytosanitary thresholds.

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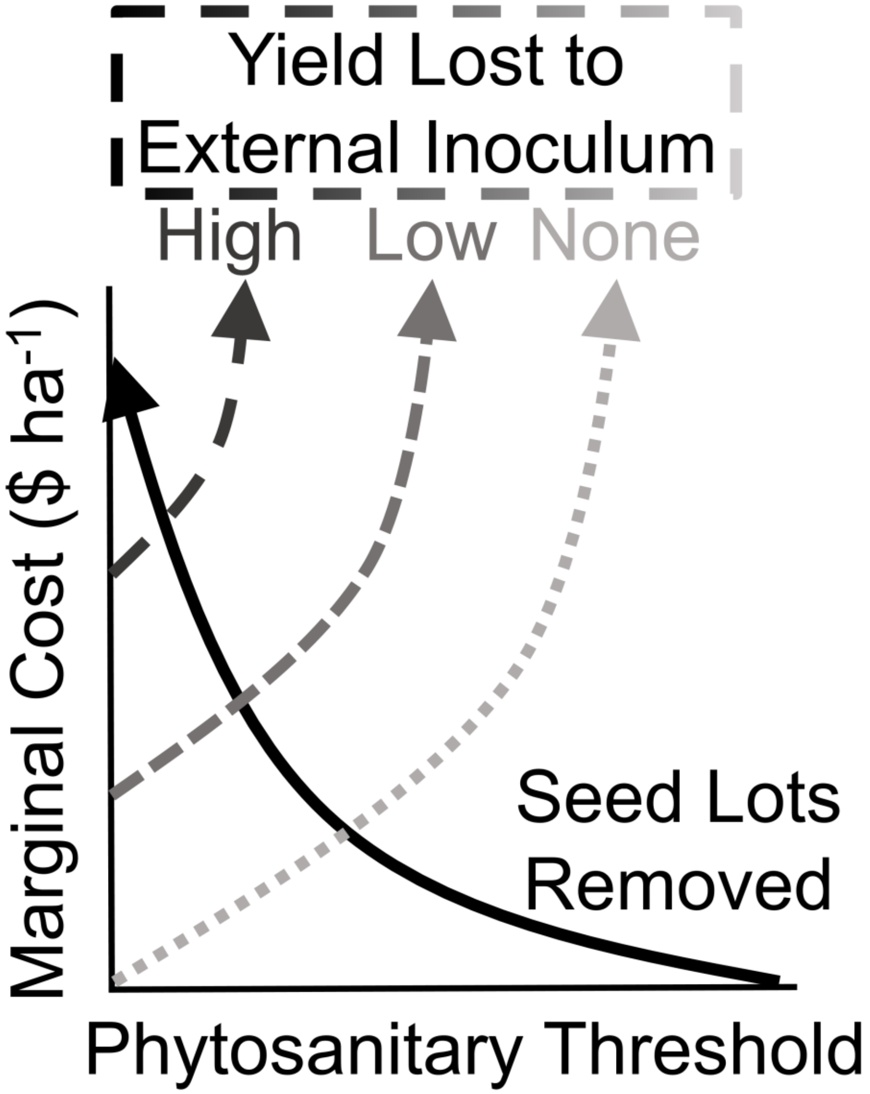

Figure 2. Diagram of the trade-off inherent in selection of a phytosanitary threshold. Higher thresholds result in a lower marginal cost of seed lot removal with strict phytosanitary thresholds and the marginal costs of yield loss depending on whether there is high, low, or no external inoculum. The threshold where the combined costs are lowest would be a candidate for optimizing profit in the short run. In the long run, more effective regional management can shift the regional inoculum level.

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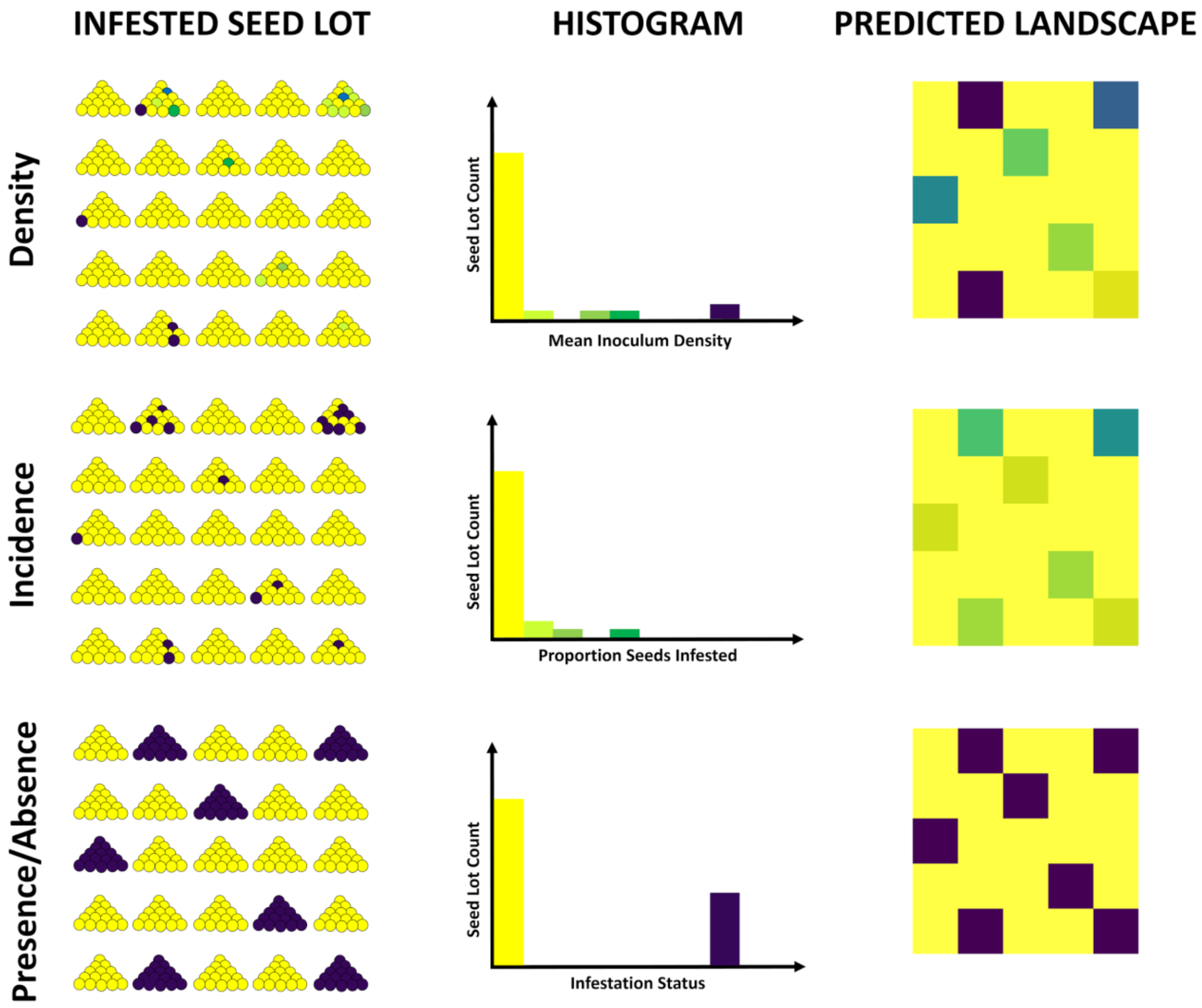

Figure 3. Effect of different assay metrics (density, incidence, presence/absence) on the perceived number of infested seed lots, and the predicted landscape if the seed lots were planted.

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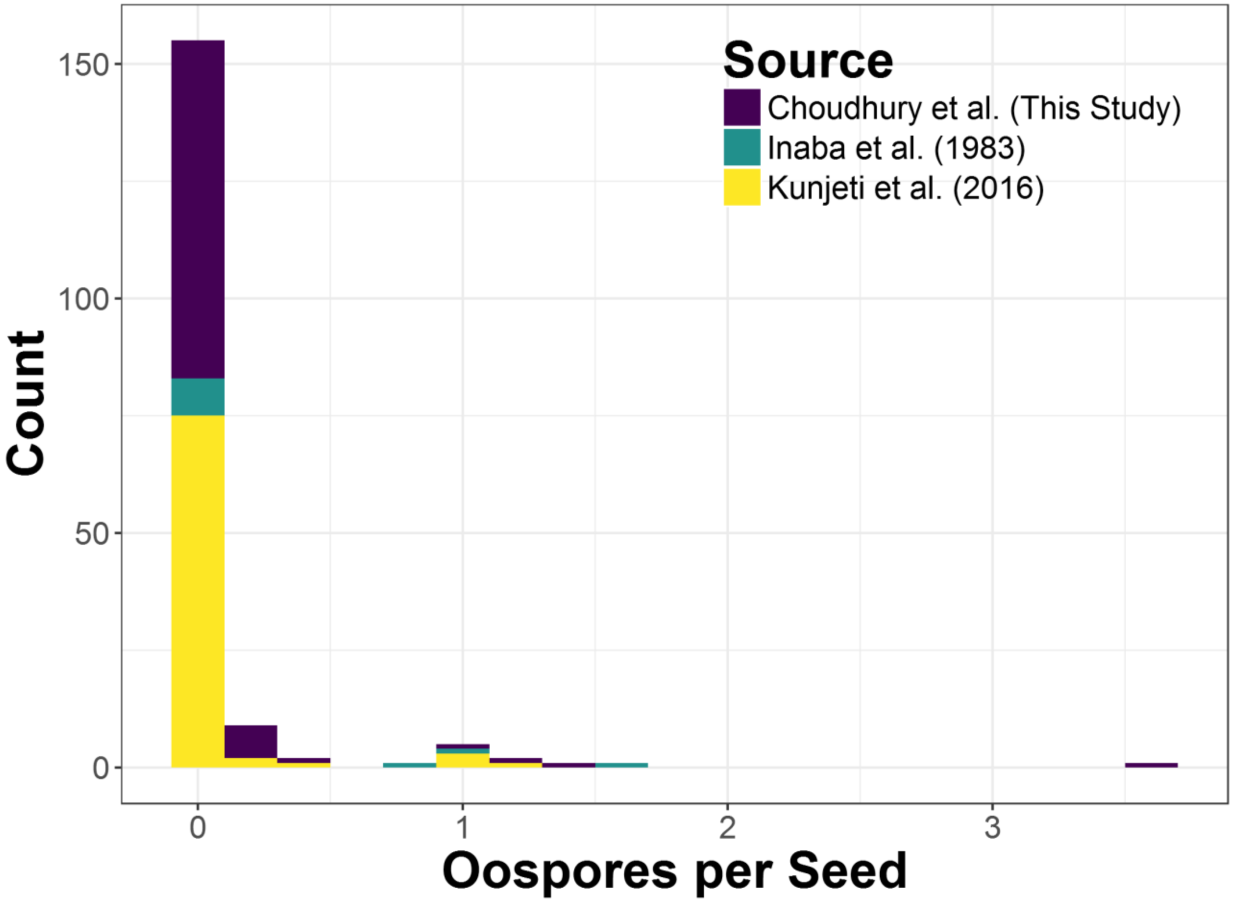

Figure 4. Histogram representing the estimated number of oospores of *Peronospora effusa* per seed found in spinach seed lots in three studies. Histogram bin width is in increments of 0.2 oospores per seed.

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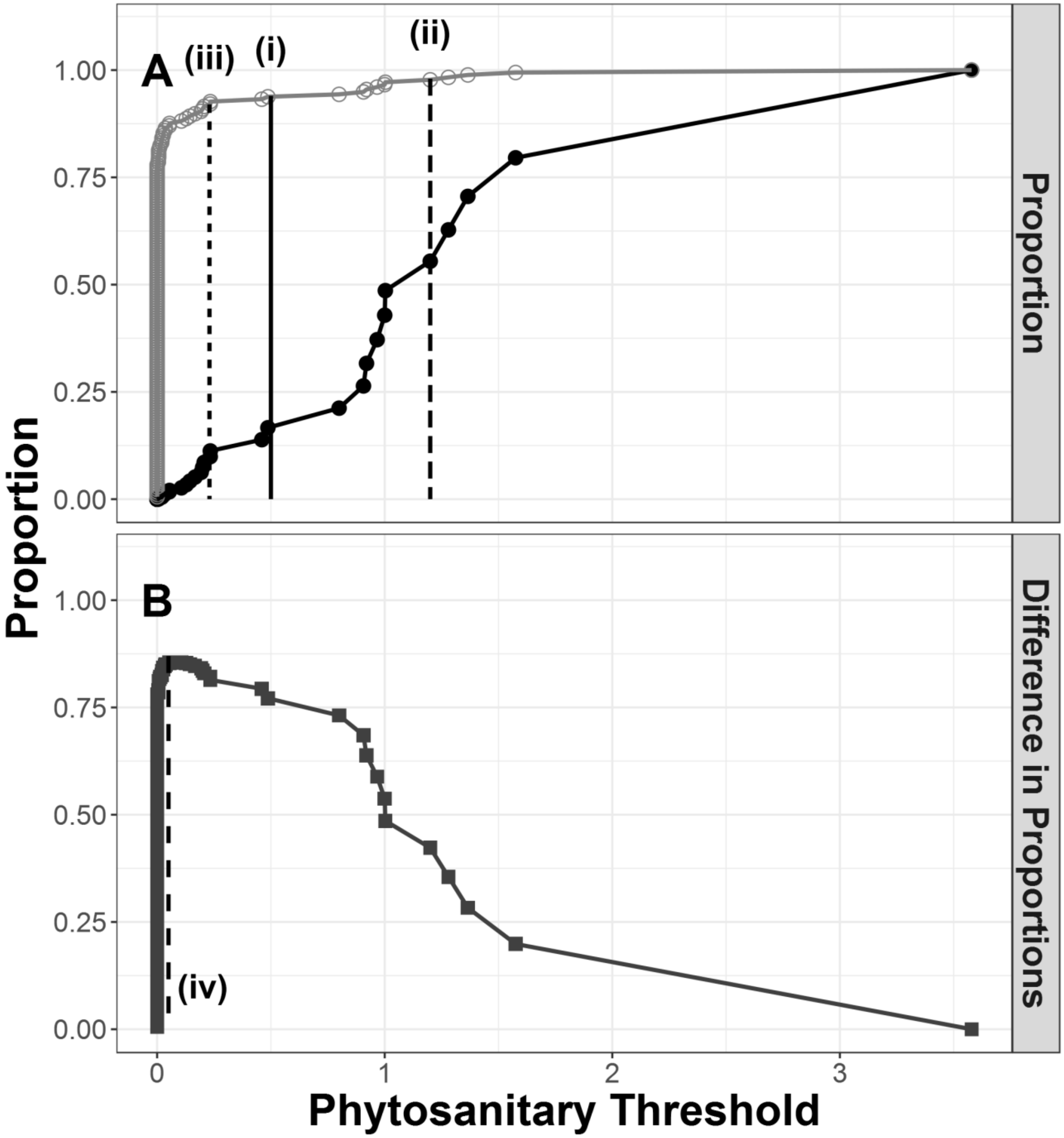

Figure 5. (A) Cumulative distributions for oospores per seed (filled circles) and seed lots (open circles) against increasing mean number of oospores per seed used as potential phytosanitary thresholds. (B) The absolute difference between the cumulative distribution curves shown in (A). Hypothetical choices for phytosanitary thresholds based on different selection criteria are indicated by vertical lines labeled (i) through (iv). (i) is an arbitrary choice for illustration, (ii) represents an

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option prioritizing seed producers, (iii) prioritizes growers and (iv) maximizes the difference between the proportion of oospores removed and the proportion of seed lots rejected. See text for a complete description.

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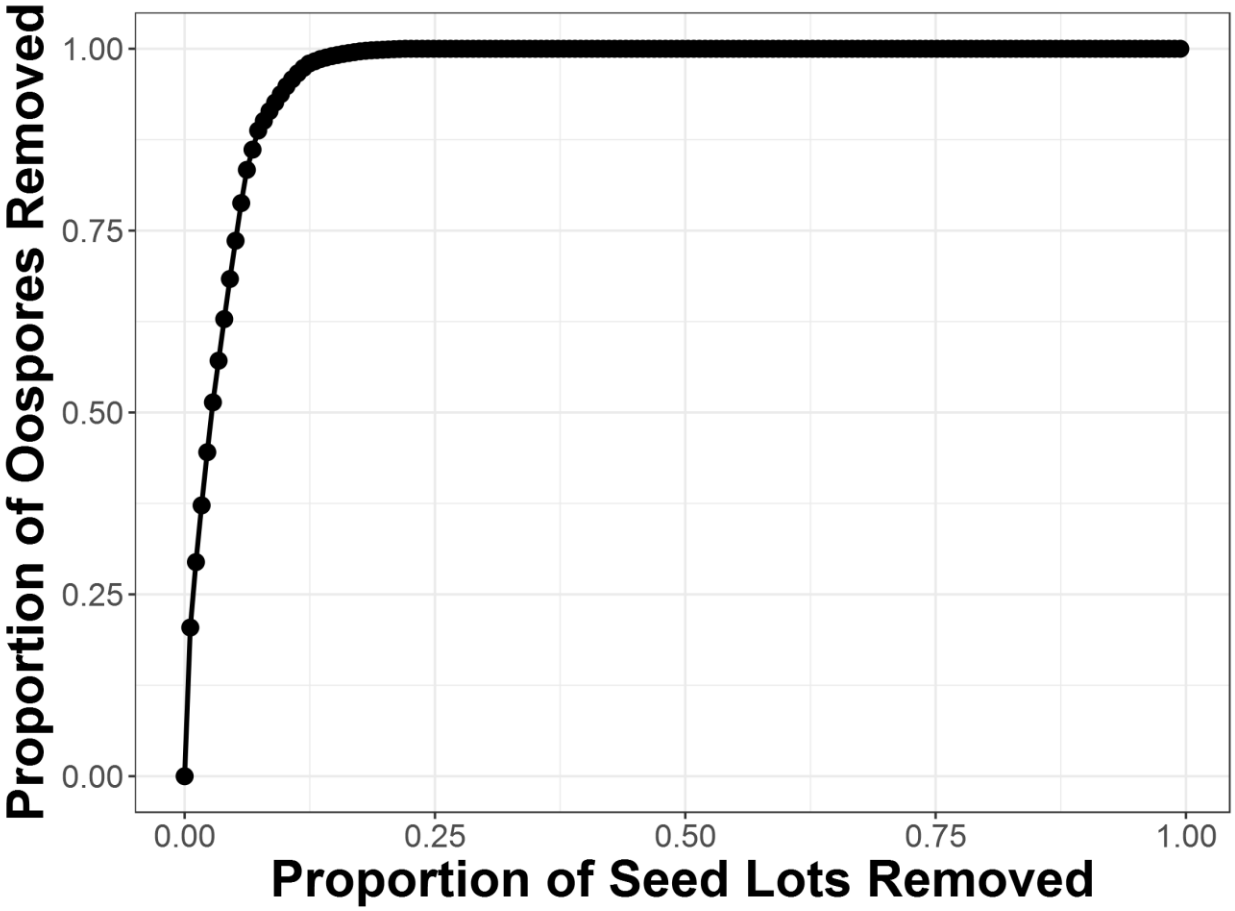

Figure 6. The relationship between the proportions of seed lots removed (rejected) and the proportions of oospores removed as a function of using the mean number of oospores per seed as a threshold for rejection. The curve is derived from the data in Figure 5. The top right corner corresponds to setting the threshold at 0 oospores/seed (all seed lots rejected, all oospores removed) and the maximum observed value, 3 oospores/seed, corresponds to the bottom left corner (no seed lots rejected, no oospores removed).

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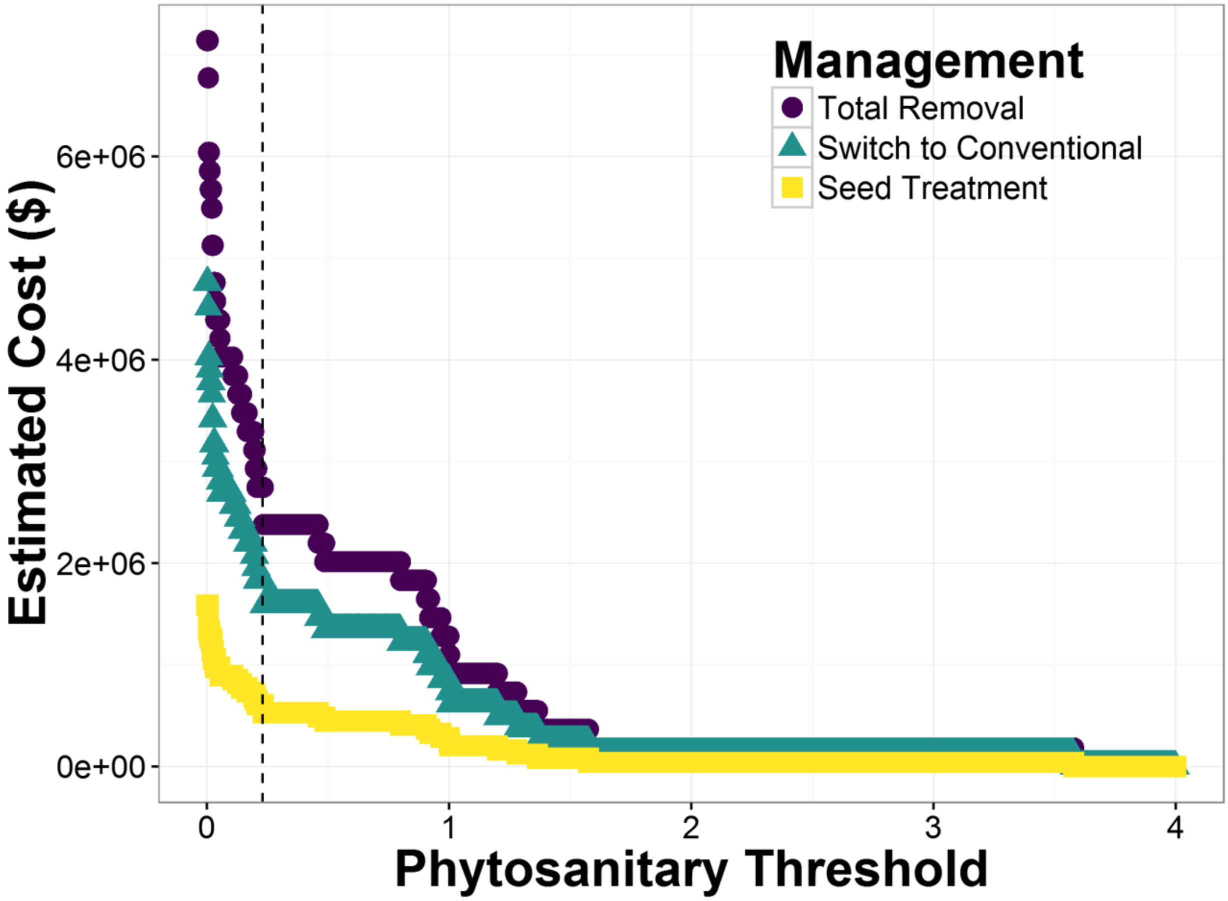
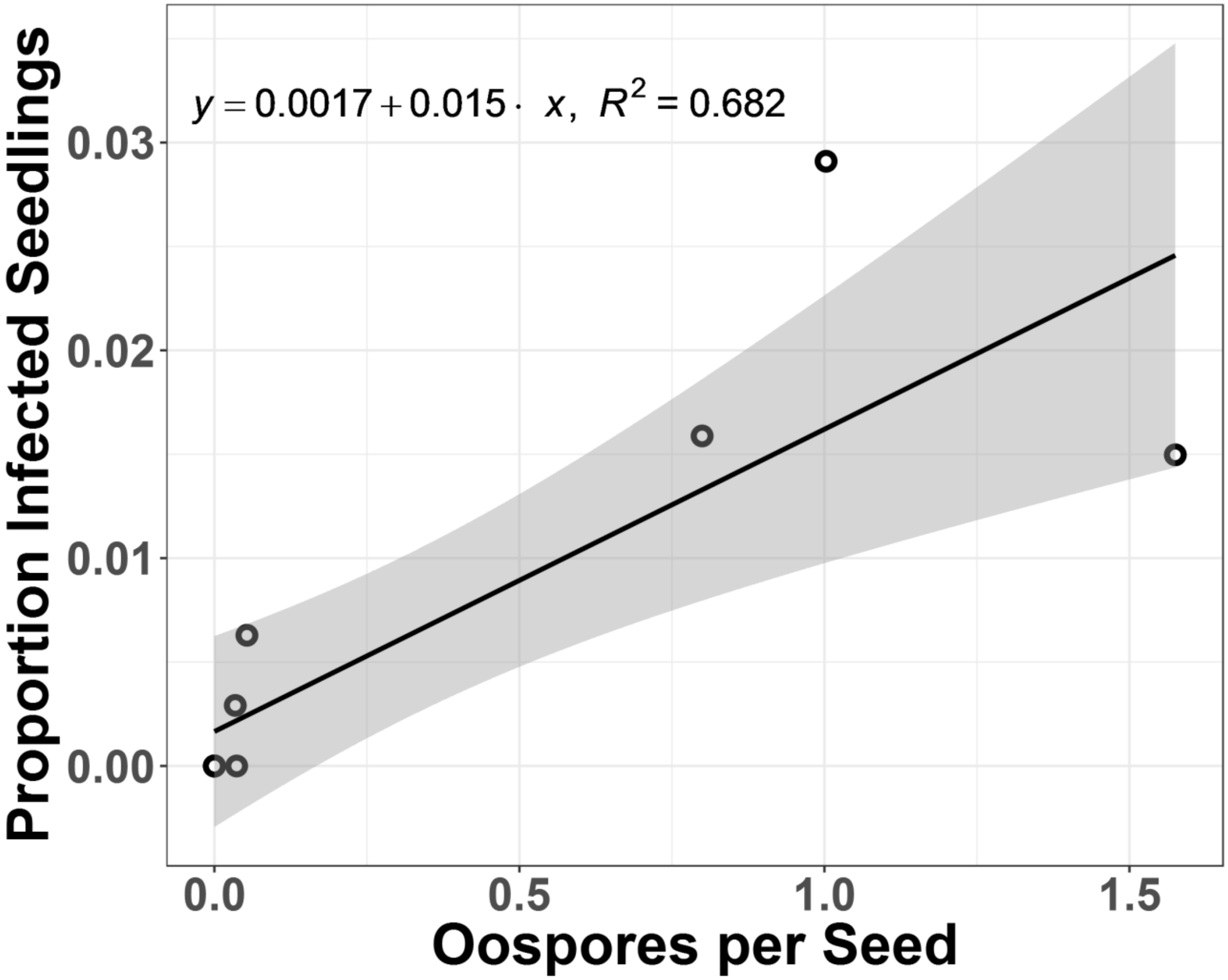


Figure 7. Outcomes from a model estimating the economic costs of implementing three management tactics across phytosanitary threshold levels of allowable oospores per seed in spinach seed lots. Management tactics are total removal of infested seed lots (purple circles), switching infested organic seed lots to conventional production (green triangles), and treating seed lots to prevent oospore success (yellow squares). Dotted vertical line indicates a threshold of 0.23 oospores per seed, which removes 90% of oospores from the affected seed lots.

UNCORRECTED VERSION USED IN INITIAL SUBMISSION TO *PHYTOPATHOLOGY*

Supplemental Table 1: Seed lot data used in this study.

UNCORRECTED VERSION USED IN INITIAL SUBMISSION TO *PHYTOPATHOLOGY*



Supplemental Figure 1: Relationship between the average number of *Peronospora effusa* oospores per spinach seed and the proportion of infected seedlings grown from the infested seed lot. Blue line represents the line of best fit of the linear regression, and the grey area represents the 95% confidence interval area. The summary equation and coefficient of determination of the linear regression are given in the upper left hand corner of the graph. Data from Table 1 of Inaba et al., 1983.

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