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# A network meta-analysis of the susceptibility of apple genotypes to powdery mildew under organic management

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ABSTRACT

The susceptibility of apple tree genotypes to powdery mildew has been quantitatively evaluated in the field by various researchers. Our aim was to attempt to overcome difficulties such as sampling error, and differences in the designs, time periods and geographical locations of studies, to form a consensus about the variation in this susceptibility over different apple genotypes under organic management by applying the methodology of meta-analysis. A systematic search of appropriate databases was conducted for relevant articles published from January 1998 to December 2017. It was required that susceptibility to powdery mildew was measured by incidence in terms of the percentage of affected leaves. Where possible, the incidence levels of the studies that satisfied given criteria were standardized using an association scheme and adjustment factors to the mean environmental conditions in what was chosen to be the base study. The literature search found 22 studies that satisfied all the inclusion criteria of the meta-analysis, and for 20 of these, incidence levels could be standardized. Since the overall virulence of the fungus varied greatly across studies, standardizing incidences was crucial. Of the 12 genotypes having the lowest mean standardized incidences in the main analysis of 94 genotypes, the least well-known are 'Earlidel', 'Florina-Quérina', 'Lourdes', 'Piros', 'Rajka', 'Topaz', '436' and '468' of which the former 6 genotypes are arguably the more appealing to producers in terms of fruit yield and the appearance, taste and texture of their fruits. Many genotypes appear to have susceptibilities relative to other groups of genotypes that are not that sensitive to changes in environmental conditions, including the native strains of the pathogen that are present, however for other genotypes this sensitivity appears to be reasonably high.

## 1. Introduction

Powdery mildew (*Podosphaera leucotricha*) is a major disease of apple trees. The fungus affects plant foliage, producing white mycelia and gradually, leaf wrinkling. In more severe cases, the disease can cause substantial defoliation and the fruit to develop russetting (Gubler and Koike, 2011). Besides the impact on the yield and quality of the fruit, frequent presence of the fungus may affect the longevity of the orchard (Marine et al., 2010).

The most common way of controlling powdery mildew is by applying fungicides, such as triazoles. However, their use can be expensive and can lead to detrimental effects on human health and the environment (Rosenberger, 2007). Also, the repeated use of fungicides contributes to the development of resistant strains of the pathogen (Borlaug and Dowsell, 2002).

The use of apple cultivars that are resistant to powdery mildew, on the other hand, may often be the most desirable method to prevent damage by the fungus, as this can avoid the disadvantages associated with other methods of control, without necessarily reducing fruit yield or the attractiveness of the fruit to the consumer.

The susceptibility of different varieties to powdery mildew may be evaluated in the field, where the incidence and severity of the native strains of the pathogen present at the site may be determined in trees exposed to natural infection. Indeed, many researchers have published their findings from these kinds of studies. Nevertheless, the way that the incidence or the severity of powdery mildew is measured varies substantially between these studies (Blazek, 2004; Bus et al., 2006 and Paz-Cuadra et al., 2014).

Moreover, even when the same measurement system is used, at least three difficulties need to be overcome to be able to adequately utilize and generalize the results of any given study. First, these results may be subject to a substantial statistical error. This is especially the case when the sample size is small, or information on the likely sampling error is not provided in the reports of these studies. Second, the virulence of the

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fungus can change substantially from one year to the next, which complicates using past results to predict outcomes in future years at the same site. Third, results obtained in one site will generally not be directly applicable to other sites, especially when these sites are in other regions or countries. These problems are compounded by the fact that the collection of genotypes that are evaluated generally differs greatly between studies, which prevents an easy corroboration of the findings.

The aim of the present study is to try to overcome the aforementioned difficulties and form a consensus about the resistance of apple genotypes to powdery mildew under organic management conditions by adapting a methodology known as network meta-analysis to an agricultural setting.

Meta-analysis represents a means by which the results of studies which attempt to address the same or similar scientific questions can be combined to obtain a clearer overview of what is currently known in the area of research concerned (Borenstein et al., 2009). The use of meta-analysis is now common in many disciplines that rely on quantitative observational or experimental data (Koricheva et al., 2013; Davis et al., 2014 and Qadir et al., 2017) and interesting extensions to the basic methodology have been proposed (Stanley and Jarrell, 1989; Sterne et al., 2002; Bowater and Escarela, 2013). More specifically, in medical research, the particular variant of meta-analysis that will be adapted for use in the present study, namely network meta-analysis (Bucher et al., 1997; Caldwell et al., 2005 and Tonin et al., 2017), has been recently recognized as being a powerful methodology for combining the results of studies in which only a small collection of the experimental units of interest are evaluated in any given one of the studies under consideration.

However, the application of network meta-analysis, or even just standard meta-analysis, has been limited in agricultural research (Fisher, 2015). Indeed, there are very distinct challenges to be confronted in applying network meta-analysis to agricultural rather than medical research. The present study will therefore attempt to break relatively new ground by exploring the benefits of network meta-analysis in addressing an issue that is of interest in horticultural disease management.

## 2. Materials and methods

### 2.1. Literature search and inclusion criteria

A systematic search of the Web of Science, PubMed and Google Scholar was conducted for articles published in the 20-year period from January 1998 to December 2017 that report experimental results of the resistance of apple tree genotypes to powdery mildew. For the searches of the Web of Science and PubMed the following combinations of words were sought in the abstracts: 'apple' and ['mildew' or 'podosphaera']. For the search of Google Scholar, the following combinations of words were sought in the title: 'apple' and ['cultivars' or 'genotypes']. Relevant papers and technical reports of the present authors were added to this collection of papers. The inclusion criteria for papers in this meta-analysis were as follows:

1) Experiments had to be conducted in the field or in a naturally ventilated greenhouse.
2) The orchard had to be organically managed, and also powdery mildew had to be allowed to develop naturally without any direct control measures.
3) Apple genotypes evaluated could not be the result of artificial genetic modification.
4) At least five different apple genotypes had to be evaluated in the study.
5) Incidence in terms of the percentage of leaves with signs of powdery mildew had to be reported.
6) Papers had to be written in English.

Multiple studies that were reported in the same paper were divided according to geographical site and year, and were then treated as separate studies. For studies that evaluated the levels of incidence in the orchard various times during the course of a year, the levels of incidence in the final evaluation were taken as being the levels of incidence for that study. Therefore, each study could only correspond to one measured incidence level for any given genotype.

### 2.2. Base study

To standardize the incidences of the pathogen, methodology used in network meta-analysis was applied. First, out of all studies identified through the meta-analysis one study was selected as the base study. This study was chosen to be the study with the highest association score. For any study, this score was calculated by summing over all apple genotypes in the study with a non-zero incidence level, the number of times each genotype appears, with non-zero incidence, in another study. A tie between studies having the same highest association score was resolved on the basis of the alphabetical ordering of the study locations.

The next step was to modify the incidences of the other studies so that they estimate what the levels of incidence would have been if each study had been conducted under the same environmental conditions, including the same atmospheric presence of the fungus, as in the base study. This was done using an association scheme.

### 2.3. Association scheme

The first study included in the association scheme was the base study. The second study included in the association scheme was the study with the most apple genotypes, with non-zero incidence, in common with the base study. The base study was therefore the 'link study' for the second study.

Subsequently, studies were included in the association scheme on the basis of which study had the highest number of genotypes, with non-zero incidence, in common with any of the studies already included in the association scheme. The study in the scheme for which this highest count was achieved was the link study for the study entering the scheme. However, if out of the studies not in the scheme, there was no study that had at least three genotypes, with non-zero incidence, in common with any of the studies already included in the scheme, but there was at least one study that had at least one genotype, with non-zero incidence, in common with the base study, then the study with the most genotypes in common with the base study was included in the scheme. The base study was therefore the link study in this case.

If, in concluding the construction of the association scheme, any study not included in the scheme had no genotypes, with non-zero incidence, in common with any of the studies in the scheme then the incidence levels for this study could not be standardized, and so the results of this study were summarized separately.

### 2.4. Determination of adjustment factors

On the basis of how studies entered the association scheme, adjustment factors for the standardization of incidence levels were calculated. The adjustment factor for a study A to its link study B in the association scheme was determined by first establishing the set of genotypes G that are evaluated in both study A and study B, and then applying the following formula:

$$\text{Adjustment factor for study A to study B} = \frac{\text{Average incidence over genotypes in set G for study B}}{\text{Average incidence over geno types in set G for study A}}$$

In keeping with the methodology of network meta-analysis, the overall adjustment factor for a study A to the base study was calculated