Forest Ecology and Management 424 (2018) 259–266

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Resistance to Cypress Canker Disease in Italian cypress has desirable effects on disease epidemiology, but may fail against novel genotypes of the pathogen *Seiridium cardinale*

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A R T I C L E I N F O

Keywords:
Clones
Cupressus sempervirens
Disease control
Epidemics
Fruit bodies
Plant breeding
Plant health
Selection
Sporulation
Strains
Susceptibility

A B S T R A C T

Prevention is hailed as the only successful and cost-effective approach to control the introduction of exotic forest pathogens, and several international policies are in place to avoid such introductions. However, once a pathogen has been introduced and is widely spread in a novel range, regulations are relaxed. Concerns have been voiced about the detrimental consequences of the introduction of multiple genotypes of a pathogen, but evidence about such consequences has rarely been documented. Cypress Canker Disease (CCD) is a pandemic lethal disease of plants in the family *Cupressaceae* caused by the fungal pathogen *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson (*Sc*). *Sc* is native to California, where populations are genetically diversified, while widely spread CCD outbreaks in the Mediterranean basin have been caused by the introduction of a single genotype. Resistant plant clones have been selected in Italy based on long-term field tests using a representative Mediterranean *Sc* genotype. Here we use a 90-day greenhouse trial on 419 21-month old grafts to test the response of four putatively resistant and three putatively susceptible clones of Italian cypress (*Cupressus sempervirens* L.) against one Mediterranean and five Californian fungal genotypes. By measuring mortality, size of the lesion caused by the fungus on the stem, and fungal sporulation, we ask the following questions: (1) Does the fast greenhouse assay confirm results of the extended field trials; (2) Is resistance confirmed for all putatively resistant clones when using a broad representation of the pathogen; and, (3) Does resistance have the potential to reduce transmission rates of the pathogen, thus potentially slowing down the CCD epidemic? Results indicated that: (1) Lesion size, but not seedlings' survival, can be used as metric to measure resistance in small grafted ramets; (2) One of four putatively resistant plant clones was considered susceptible at the end of the trial; and, (3) Sporulation was significantly lower in resistant than in susceptible plant clones. Based on these results, we conclude that resistance may be a viable disease control approach in this pathosystem due to the effect it has on both plant survival and fungal sporulation. However, we also conclude that resistance can be considered robust and effective against the pathogen species in its entirety, only if tested using several fungal genotypes. This study is one of the first to show that multiple introductions of a pathogen could break down host resistance and have a measurable detrimental effect on native ecosystems, suggesting that international policies should be revised accordingly.

1. Introduction

A pressing current aim of both plant health organizations and of the scientific community at large is to increase vigilance measures about: (i) the prevention of the introduction of exotic pathogens into a new region or country; and, (ii) the eradication or spread control of newly introduced plant pathogens. Conversely, less regulatory attention is given to diseases that are already established in a territory. This approach fails to take into account the possibility that different genotypes or strains, and/or different lineages of the same pathogen species may be further introduced causing clearly distinct outbreaks. This is currently a distinct possibility for diseases caused by different lineages of the same emergent pathogen (Grünwald et al., 2012; Eyre et al., 2014) or for pathogens that are currently evolving allopatrically after their

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https://doi.org/10.1016/j.foreco.2018.05.008
Received 3 March 2018; Received in revised form 24 April 2018; Accepted 3 May 2018

introduction in different parts of the world (Oswald et al., 2012). The occurrence of new outbreaks of emerging plant diseases caused by multiple introductions is a crucial issue, given we live in an era of increasing international movement of plants and of their associated pathogens (Ghelardini et al., 2017). The relevance of this issue for exotic and invasive plant pathogens should not be surprising, as it has been proven over and over again that biological invasions are greatly favored by multiple introductions (Facon et al., 2008).

The same concerns arise when dealing with different but closely related, morphologically undistinguishable, and interfertile species. Introductions of new and closely related species, in fact, can lead to taxa with greater invasive potential, through hybridization with a sexually compatible resident pathogen (Brasier, 2000; Brasier et al., 2004; Gonthier et al., 2007; Newcombe et al., 2000; Santini et al., 2005). The migration of different genotypes or of different mating types of a pathogen in areas where the species is already present, can also lead to sexual reproduction resulting in more aggressive genotypes strains that may alter the sensitivity of pathogens to specific compounds used for chemical control or may be able to infect plant genotypes selected for resistance (Goodwin et al., 1998; Singh et al., 2011). Both scenarios can cause extremely devastating new waves of invasion.

Cypress canker disease (CCD) is a destructive disease causing crown dieback and mortality in several plant species of the family *Cupressaceae*. The first epidemic was reported in 1928 in California (USA) on Monterey cypress (*Cupressus macrocarpa* Hartw. ex Gordon) (Wagener, 1928), Since this initial report, the disease spread across the globe (Graniti, 1998), being transported among continents by the trade of ornamental plants (Danti et al., 2014; Della Rocca et al., 2011b) and becoming a pandemic within fifty years. Epidemics were particularly severe in California on *C. macrocarpa,* and around the Mediterranean Basin on *Cupressus sempervirens* L., and caused significant losses in woods, plantations, gardens, parks and nurseries, also endangering the iconic role held by cypresses in the Mediterranean landscape (Danti et al., 2013; Danti and Della Rocca, 2017). However, the reasons for the severity of the two outbreaks in California and in countries of the Mediterranean basin are diametrically opposite. In California, the pathogen is native and causes no disease on endemic cypress species in their natural range (Della Rocca et al., 2011a, 2013). The epidemic was and in part is still caused instead by the massive planting of coastal Monterey cypress in agricultural lands of inland valleys, characterized by very different ecological conditions than coastal sites (Danti and Della Rocca, 2017). In countries around the Mediterranean basin, the CCD causal agent *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson is exotic and the entire outbreak was caused by the introduction of a single genotype rapidly adapting to the new ecosystem and to the new host, *C. sempervirens* (Della Rocca et al., 2011a, 2013). In both regions, disease incidence is considerably augmented by the presence of the extremely susceptible artificially created hybrid species Leyland cypress (x *Hesperotropsis leylandii* (A.B. Jacks. & Dallim.) Garland & Gerry Moore) (Danti et al., 2014; Danti and Della Rocca, 2017).

Three main outcomes stemmed from the genetic analysis of California and Mediterranean populations of the fungus (Della Rocca et al., 2011a, 2013): (i) in California, the *S. cardinale* population is genetically diversified and includes two clearly distinct, albeit intermixing, metapopulations, called β-tubulin haplotype A (β-tubA) and β-tubulin haplotype B (β-tubB); (ii) the Mediterranean outbreak was due to a single introduction of a β-tubA genotype; and, (iii) sexual reproduction is occurring in California, whereas the European population has been generated solely through clonal reproduction.

As a result of the intrinsic difference between the two outbreaks, their respective trajectories have been substantially different. In California, disease is controlled by: (a) avoiding planting coastal cypresses inland (inland cypress species are per se quite disease-resistant), and, (b) avoiding the planting of Leyland cypress. In the Mediterranean region, due to the exotic nature of the pathogen, susceptibility of native *C. sempervirens* was initially very high and CCD affected up to 70–90% of trees in some conducive sites during the ‘70-‘80 decades (Panconesi, 1990; Graniti, 1998). Currently, CCD in the Mediterranean is managed with an integrated approach that includes implementing of extinctive measures (sanitation), using resistant cypress selections for new plantations, avoiding off-site plantings and extensive use of Leyland cypress; and, finally, the use of preventive chemical treatments (only for very high value individual trees) (Della Rocca et al., 2011c). However, where the use of Italian cypress remains a priority, the employment of the cultivars selected for their resistance to CCD is probably the most valuable approach (Danti et al., 2006). These cultivars were developed in the last 35 years through a genetic improvement program conducted in Italy by the IPSP-CNR, (Raddi et al., 1984; Xenopoulos, 1990; Santini and Di Lonardo, 2000; Danti et al., 2011).

Screening cypress candidates for resistance has relied on execution of artificial inoculations on a fair number of ramets of each cypress clone, planted in contrasting sites to estimate the clone and the environment effects on the host response. Italian cypress shows a polygenic, or quantitative resistance to CCD and its phenotypic expression is influenced by both the genotype and environment. For this reason, the assessment of the resistance stability is carried out in parallel in different pedo-climatic environments (Danti et al., 2013). This horizontal (partial) resistance of cypress to CCD is known not to be a universal trait, as, similarly to a dam, it can be overcome by more virulent strains of the fungus or by multiple close infections ((Panconesi, 1990) As pathogenicity tests showed repeatedly low variability in virulence among the Mediterranean isolates of the fungus (Raddi and Panconesi, 1984; Ponchet et al., 1990; Moricca et al., 2000; Pedron et al., 2007), for many years the same *S. cardinale* isolate (ATCC 38654) has been used in a standardized selection procedure. The validity of this approach was in part supported by a study showing that the entire pathogen population of Southern Europe is derived from the introduction of a single genotype (Della Rocca et al., 2011a). However, two further studies (Della Rocca et al., 2013; Garbelotto et al., 2015) have indicated exotic pathogen populations in the Mediterranean have evolved and adapted, and at least two genetically and phenotypically distinct subpopulations can now be identified in the Mediterranean basin.

Hence, a key and largely unanswered question is whether cypress cultivars previously selected for resistance to CCD in Italy based on a single pathogen genotype are also resistant when challenged with genetically different isolates of *S. cardinale*. The two main aims of this study, thus, were: (1) To test a fast assay to explore the response of Italian cypress clones previously defined as resistant or susceptible to a larger sample of pathogen genotypes, representative of the diversity in native pathogen populations; (2) To study the correlation between resistant/susceptible plant phenotypes, lesion size, mortality rate, plant stem diameter, and sporulation, using several plant clones and pathogen genotypes.

2. Materials and methods

2.1. Experimental design

A total of 419 plants of Italian cypress (*Cupressus sempervirens*) representing four putatively resistant (R) and three putatively susceptible (S) clones to CCD were used in this experiment. Plants belonging to a clone were thus genetically identical, but each was grafted on commonly used 1-year-old seed rootstock, and grown for 18 months in 0.35-liter free draining pots containing peat, compost, and perlite (3:1:1 v/v/v) in Italy. Plants were shipped to the USA, and, after the necessary State and Federal inspections, they were transferred for three months to a containment greenhouse at the University of California, Berkeley. Plants were artificially inoculated on June 15th 2016 using six *S. cardinale* isolates, when they were 21 months old.

The seven cypress clones are currently maintained in the IPSP-CNR clonal collection, and were chosen based on their response to *S. cardinale* inoculations, following a standard procedure developed by the