Inoculum and Disease Dynamics of Circular Leaf Spot of Persimmon Caused by *Mycosphaerella nawae* Under Semi-Arid Conditions

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Abstract

The epidemiology of circular leaf spot of persimmon, caused by *Mycosphaerella nawae*, was studied in a semi-arid area in Spain for two consecutive years. No conidia were observed on diseased leaves and all infections were thought to be caused by ascospores formed in the leaf litter. Ascospores were released mainly in April and May, but relatively low numbers in June were able to induce severe symptoms on trap plants. Temperature was not significantly correlated with ascospore catches or disease incidence on trap plants, indicating that it was not a limiting factor for disease development during the period of study. Rainfall was above normal, but still considerably lower than in endemic areas of Korea. Most infections coincided with rains, but the disease was observed also on trap plants exposed to less than 1 mm of precipitation and even in the absence of rain. Orchards were flood irrigated once inoculum deposits in the leaf litter had already been depleted, so it was not possible to determine its effects on ascospore release and disease development. The use of a wind tunnel to determine inoculum potential allowed detection of physiologically mature ascospores of *M. nawae* in the leaf litter 1-2 weeks before they were released to air in the orchard. Disease progress was fitted

to the monomolecular growth curve, associated with monocyclic pathogens and diseases with a variable incubation period as a function of the host phenology.

Keywords *Diospyros kaki,* Mediterranean climate, risk analysis, dew

Introduction

Global production of persimmon (*Diospyros kaki* L. f.) is estimated at about four million tonnes, with a cultivated area of 785,000 ha (FAO, 2009). Far East Asia is considered the centre of origin of this fruit tree (Badenes et al., 2003), and currently China, Japan and Korea represent more than 95% of the total production worldwide (FAO, 2009). Although there are reports of persimmon trees in Spain from the XVI century (Giordani, 2003), cultivation of this crop expanded significantly during last decade due to the extensive planting of the cultivar 'Rojo Brillante', coupled with the development of postharvest treatments to remove astringency without reducing fruit firmness (Arnal and Del Río, 2003).

Circular leaf spot disease of persimmon, caused by *Mycosphaerella nawae* Hiura & Ikata, is widespread in persimmon-growing areas of Japan and Korea (Ikata and Hitomi, 1929; Kang et al., 1993). In 2008, the disease was detected in the Mediterranean Basin in Valencia Province in east-central Spain, which was the first report in a semi-arid area (Vicent, 2008; Berbegal et al., 2010). The disease causes necrotic spots on leaves, chlorosis and early defoliation. Although *M. nawae* is a foliar pathogen, leaf lesions and defoliation induce premature fruit maturation and abscission, resulting in serious economic losses (Kwon and Park, 2004; Berbegal et al., 2010).

The fungus reproduces in pseudothecia formed in leaf litter. Ascospores mature in spring and are released to the air when specific temperature and moisture conditions are met. In epidemiological studies conducted in Korea, temperatures over 15ºC and rain were the main factors associated with ascospore discharge (Kang et al., 1993; Kwon et al., 1995; 1997a).

Under laboratory conditions, temperatures above 10ºC and at least 1 mm of precipitation were necessary for release of significant numbers of ascospores (Vicent et al., 2011). In Korea, infection occurs mainly in the spring and early summer (Kang et al., 1993; Kwon and Park, 2004), but the specific temperature and moisture requirements for infection have been not determined. Secondary inoculum consisting of *Ramularia*-type conidia was described in Korea. These spores are produced on leaf lesions and are capable of infecting and inducing symptoms on leaves. However, their role in disease epidemics is considered generally less important than ascospores (Kwon et al., 1998a; Kwon and Park, 2004). Circular leaf spot is characterized by a long incubation period, with a lag time between infection and symptom expression of up to four months (Kwon and Park, 2004).

Several fungicide applications per season are required for the economic control of circular leaf spot (Kwon et al., 1997b; Berbegal et al., 2011). However, their efficacy is extremely dependent on the synchrony between spray timing and infection periods in each region. Persimmon-growing areas in southern Korea and Japan are characterized by humid-subtropical climate (*Cwa*), with a summer rainfall pattern and yearly precipitation around 1,500 mm (WMO, 2011). In these areas, persimmon trees can be grown under rainfed conditions. In contrast, the climate in Spain is typically Mediterranean (*Csa*), with dry summers and annual precipitation rarely over 500 mm, distributed mainly in spring and fall (WMO, 2011). In semi-arid areas such as Spain, persimmon cultivation is only possible with irrigation. Due to the climatic and agronomic differences between two areas, information about circular leaf spot epidemiology and infection periods from Korea cannot be extrapolated to Spain. In fact, based on classic climatic comparisons, the emergence and severe impact of the disease in a semi-arid area were completely unexpected (Makowski et al., 2011). Therefore, the basic epidemiological traits of circular leaf spot under semi-arid conditions and its associated adaptive mechanisms need to be determined.

The objectives of this study were: (i) to study the dynamics of inoculum potential and inoculum availability of *M. nawae* in affected orchards, and (ii) to determine the infection periods and disease progress of circular leaf spot under semi-arid conditions in Spain.

Materials and methods

Experimental orchards

Experiments were conducted in 2010 and 2011 in four commercial persimmon cv. Rojo Brillante orchards severely affected by the disease at Benimodo, L'Alcúdia, Guadassuar and Villanueva de Castellón in Valencia Province, Spain. Orchards were 6 yr old at Benimodo, 11 yr old at L'Alcúdia, and 9 yr old at Guadassuar and Villanueva de Castellón. Trees were grafted on *D. virginiana* L. rootstock at Benimodo, and on *D. lotus* L. at Villanueva de Castellón, Guadassuar and L'Alcúdia. All four orchards were flood irrigated, with rows oriented east to west and on a 4 × 5-m tree spacing.

Isolations from affected leaves in the canopy and leaf litter were performed to verify the presence of the pathogen in the orchards prior to the experiments. Symptomatic green leaves were surface disinfested with 1% NaOCl for two min and small fragments from necrotic lesions were plated in Potato Dextrose Agar (PDA) amended with 0.5 g L^{-1} streptomycin sulphate (PDAS). Isolations from leaf litter were carried out by and attaching wetted leaf pieces to the top of a Petri dish and allowing the ascospores to be ejected from pseudothecia onto PDAS. Plates were incubated at 24°C in the dark and examined daily during two weeks. The resulting fungal colonies were transferred to PDA to characterize colony morphology. Molecular identification was performed on five representative isolates from each orchard by sequencing the internal transcribed spacer (ITS) using the conserved primers ITS1 and ITS4 (White et al. 1990).

In the centre of each orchard, an experimental area of ≈0.2 hectares (10 × 10 trees) remained untreated during the 2-yr period of study. Environmental data were monitored hourly in the orchard at Benimodo with an automated meteorological station (Hobo U30, Onset Computer Corp.) including sensors for temperature and relative humidity (Hobo S-THB, accuracies ± 0.2ºC, ± 2.5%), rainfall (7852, Davis Instruments Corp, resolution 0.2 mm) and leaf wetness duration (Hobo S-LWA, resolution 0.59%). Environmental monitors were located within the row in the experimental area at the site of a missing tree. Data were collected at 1.5 m above the soil surface, in the top one-quarter of the canopy height. Leaf wetness sensors were placed with a northerly exposure and fixed at a 30-degree angle from the horizontal. Leaf unfurling (BBCH 15) was observed in the orchards on 19 April in 2010 and 7 April in 2011 (García-Carbonell et al., 2002). Full flowering (BBCH 65, 50% of flowers open) was observed on 13 May in 2010 and on 3 May in 2011.

Inoculum dynamics and infection periods

Inoculum dynamics and infection periods were studied from March to September in all four orchards in 2010 and 2011. The dynamics of inoculum potential in the leaf litter were studied by covering dry leaves on the experimental area in each orchard with a plastic mesh (2 x 2 m, 5-by-5-mm openings) fixed with four stainless-steel pins. Leaf litter density under the plastic nets was adjusted to ≈350 g of dry leaves m^{-2} (Vicent et al., 2011). A sample of 20 g of dry leaves was collected weekly in each orchard and soaked for 15 min in distilled water. Immediately after soaking, leaves were placed with the abaxial surface facing upward in a wind tunnel for 30 min until they were visibly dry (Whiteside, 1973; Vicent et al., 2011). During the process, air and water temperature was maintained at ≈21°C. Released ascospores were collected on a glass microscope slide (26 x 76 mm) coated with silicone oil (Merck). Spores were stained with lactophenol-acid cotton blue and examined at 400X magnification. All ascospores showing the

morphological characteristics of *M. nawae*; spindle-shaped, 10-13 x 3-4 µm, hyaline, 2-celled with a medium or slightly supramedian septum (Kwon et al., 1998b), were counted in four microscope field transects.

The dynamics of airborne inoculum were studied by placing four glass microscope slides coated with silicone oil in the centre of the experimental area in each orchard. Slides were placed under a plastic rain shelter (0.3 x 0.3 m) 0.25 m above the soil surface at a 45-degree angle from the horizontal, covering the four cardinal points (Campbell and Madden, 1990). Microscope slides were changed weekly and ascospores of *M. nawae* were counted as described above.

The presence on *M. nawae* conidia on leaf lesions was evaluated from first symptom appearance to complete leaf fall in both years. Samples of 25 affected leaves on the canopy and recently fallen on the orchard floor were collected weekly in each orchard. Leaf lesions were observed under the stereomicroscope at 40X magnification to find fungal structures, which were then mounted on glass slides and examined at 400X for identification.

To determine the distribution of infection periods, sets of three trap plants were placed in centre of the experimental area at Benimodo orchard each week. Trap plants were 2-yr-old 'Rojo Brillante' persimmon trees grafted on *D. virginiana* rootstock maintained in a greenhouse at the IVIA research station in absence of inoculum. Plants were grown in plastic pots (250 mm in diameter by 200 mm deep) containing potting mix (75% peat, 25% sand, vol/vol) until leaves were fully developed. Exposed trap plants were returned to a screenhouse and disease severity was evaluated periodically on all leaves using the following severity rating scale: 0 = no lesions observed; 1 = less than 10 lesions; 2 = 10 to 20 lesions; 3 = more than 20 lesions; 4 = defoliated. Defoliation was assessed by counting the number of nodes on each shoot from which leaves had abscised. Disease incidence was calculated considering the total percentage of symptomatic and defoliated leaves. Correlations among weather variables, inoculum potential, airborne inoculum, and disease incidence on trap plants were analysed using the CORR

procedure in SAS 9.0 (SAS Institute, Cary, NC). In each weekly period, inoculum potential on the first day and accumulated ascospores catches on the last day were evaluated.

Disease progress

Disease severity was evaluated periodically in four two-tree plots randomly selected in the experimental area in Benimodo and L'Alcúdia orchards. Evaluation dates in 2010 were 14, 17, 19, 22, 25 September, 3, 10, 17, 25 October in Benimodo, and 7, 10, 13, 16, 19, 27 September, 3, 10, 18 October in L'Alcúdia. Evaluation dates in 2011 were 28, 31 August, 7, 13, 19, 25 September, 5 October in both orchards. All leaves on 10 shoots arbitrarily selected in each tree (≈ 70 leaves $tree^{-1}$) were rated according to severity scale described above. Disease incidence was calculated considering the total percentage of symptomatic and fallen leaves. Disease growth models were evaluated by nonlinear regression of disease incidence data against days after disease onset using the NLIN procedure in SAS 9.0.

Results

Inoculum dynamics and infection periods

Fungal colonies isolated from affected leaves and leaf litter in the experimental orchards were dark grey to black, erumpent, with sparse aerial mycelium and a characteristic slow growing pattern. The ITS sequences from all isolates analysed had 100% identity with the GenBank accession nº GQ465767 of *M. nawae*.

No conidia were observed on leaf lesions in either 2010 or 2011. Airborne ascospores of *M. nawae* were detected in 2010 from 25 March to 22 July in Benimodo, Guadassuar and Villanueva de Castellón orchards (Fig. 1). In L'Alcúdia orchard, ascospores were detected from

31 March to 22 July. More than 81% of total airborne ascospores were detected during April and May. Ascospores were discharged artificially from leaf litter samples collected from 18 March to 29 July in all four orchards. Ascospores released from the leaf litter in April and May represented more than 83% of the total collected during the period of study.

In 2011, airborne ascospores were detected in the orchards from 29 March to 19 July (Fig. 1). Ascospore catches during April and May represented more than 90% of the total. Ascospores were discharged artificially from the leaf litter samples collected from 22 March to 30 August. The percentages of released ascospores in April-May and June-July were 42.3% and 49.7% of the total in Benimodo, 72.2% and 22.3% in L'Alcúdia, 43.7% and 50.1% in Guadassuar, and 54.9% and 35.2% in Villanueva de Castellón.

Trap plants in all exposure periods from 25 March to 8 July in 2010 and from 5 April to 21 June in 2011 were affected by the disease (Fig. 2). The percentage of affected leaves ranged from 1.8% to 92.9% in 2010 and from 10.1% to 65% in 2011.

In 2010, average weekly temperature ranged from 8ºC to 27.1ºC (Fig. 2). Average leaf wetness duration ranged from 4.7 h day^{-1} to 16 h day^{-1} and relative humidity ranged from 45.8% to 84.4%. A total of 208.8 mm of rain and 24 rain days (>1 mm) were recorded from March to June. In 2011, average weekly temperature ranged from 8.3ºC to 26.1ºC. Average leaf wetness duration ranged from 4.9 h day^{-1} to 19.1 h day^{-1} and relative humidity ranged from 41% to 80.3%. A total of 245.3 mm and 25 rain days were recorded from March to June.

In 2010, a significant positive correlation ($P < 0.01$) was observed between inoculum potential in the leaf litter and airborne ascospores in all four orchards, but only in L'Alcúdia in 2011 ($P < 0.05$) (Table 1). In 2010, inoculum potential in the leaf litter was significantly negatively correlated ($P < 0.01$) with relative humidity. The number of airborne ascospores was significantly positively correlated ($P < 0.05$) with rainfall in 2011. In both years, disease incidence on trap plants was significantly positively correlated with inoculum potential in the leaf litter and airborne ascospores, and negatively correlated with relative humidity.

Significant relationships were identified also among environmental variables. A positive correlation ($P < 0.01$) was detected between rainfall and rain days. Both variables were significantly negatively correlated with temperature and positively correlated with leaf wetness in 2010 and 2011. Temperature was significantly negatively correlated ($P < 0.01$) with leaf wetness only in 2011. In 2010, relative humidity was positively correlated ($P < 0.01$) with leaf wetness.

Disease progress

In 2010, the first symptoms of the disease were observed on 10 September, 120 days after full flowering (DAFF), in L'Alcúdia, and on 17 September (127 DAFF) in Benimodo. In 2011, the first symptoms were observed on 31 August (120 DAFF) in both orchards. Disease progress curves were best described by the monomolecular model:

$$inc = 1 - (1-y_0) \exp(-r\,T) \qquad (1)$$

where inc = disease incidence, y_0 = initial disease increase, r = rate of disease increase, and T = time in days (Fig. 3). Estimated values of initial disease increase ranged from 0.1114 in Benimodo in 2011 to 0.2024 in 2010, but no significant differences were observed among them. The rate of disease increase ranged from 0.0855 in Benimodo in 2011 to 0.2652 in 2010, which was significantly higher compared to the other values. Relative mean square errors (*RMSE*) ranged from 0.0271 to 0.0612. *Pseudo*-R^2 values were higher than 0.99 and $P < 0.0001$ in all cases (Table 2).

Discussion

Ascospores of *M. nawae* were released from the end of March to middle July, but most of them were captured in April and May. In southern areas of Korea, ascospores were released from

early May to middle August, although maximum dissemination was generally from early June to mid-July (Kang et al., 1993; Kwon et al., 1995; Kwon and Park, 2004). Average monthly temperatures from January to July in Valencia are 3.6ºC higher than in southern regions of Korea (WTO, 2011). As with host phenology (George et al., 1994), higher temperatures may influence ascocarp development accelerating ascospore maturation and release.

Recent work established the temperature threshold for ascospore release at about 10ºC (Vicent et al., 2011). In this present study, temperature was above this value from 16 and 8 March onwards in 2010 and 2011, respectively. This environmental variable was not significantly correlated with airborne ascospore counts, indicating that it was not a limiting factor for ascospore release during the experimental period.

Field studies conducted in Korea indicated that rain was strongly associated with the presence of airborne ascospores (Kang et al., 1993; Kwon et al., 1995; 1997a). Under laboratory conditions, at least 1 mm of precipitation was necessary to release significant numbers of ascospores (Vicent et al., 2011). Although rainfall was positively correlated with ascospore catches only in 2011, measurable amounts of rain were recorded in 12 of the 16 weekly periods with airborne ascospores in 2010. Statistical significance was probably affected by the presence of rains before and after the period of inoculum availability. Relatively low numbers of ascospores were detected in five weekly periods without rain or irrigation. Although these ascospores were probably discharged during the last days of the preceding period, further studies in dry years would be necessary to clarify the possible effect of dew in ascospore release (Vicent et al., 2011).

The spore trap used was similar to that of the epidemiological studies conducted in Korea, and the results obtained were consistent with the known biology of the pathogen. However, stronger relationships with environmental variables might be detected using spore traps with higher time resolution and improved collection efficiency (Jackson and Bayliss, 2011).

In 2010, inoculum potential in the leaf litter was correlated positively with airborne ascospores in all four orchards, but only in L'Alcúdia in 2011. Measurable levels of inoculum potential were detected before and after the period of ascospore release, possibly influencing the statistical output. Similar relationships between inoculum potential in the leaf litter and airborne ascospores have been described for other ascomycetes (Luley and McNabb, 1989; Aylor, 1996; Kollar, 1998).

The discharge test allowed detection of physiologically mature ascospores of *M. nawae* in the leaf litter 1-2 weeks before they were released to air in the orchard. Even considering the same weekly period, inoculum potential data were available for the first day whereas airborne ascospores were counted the last day. Therefore, this technique offers some possibilities to predict the onset of ascospore release and forecast subsequent infection periods. It could be also further developed in potential ascospore dose studies (Gadoury and MacHardy, 1986) and combined with ascocarp maturation models (Kim, 2007).

Previous studies suggested that flood irrigation could increase the rate of disease progression by favouring ascospore release (Vicent et al., 2011). Accumulated precipitation from March to June was 208.8 mm and 245.3 mm in 2010 and 2011, respectively. These values are greatly above the normal value of 129 mm (WMO, 2011), so irrigation in the study area was scheduled from middle July onwards, when inoculum in the leaf litter had already been depleted. Further studies in dry years would help to clarify the role of flood irrigation in circular leaf spot epidemics. In any case, persimmon growers in Spain are increasingly moving to drip irrigation.

Infection periods on trap plants were detected from 25 March to 8 July in 2010 and from 5 April to 21 June in 2011, with a duration of 105 and 77 days, respectively. Experimental orchards had unfurled leaves on 19 April in 2010 and 7 April in 2011, so infection periods on the trees were likely shorter than on trap plants. In epidemiological studies conducted in Korea from 1992 to 1996, infection periods on trap plants started in mid-May and lasted until the end

of July with a duration of 60-81 days (Kang et al., 1993; Kwon and Park, 2004). Differences in infection periods between Spain and Korea would be associated with the patterns of inoculum availability in each geographical area.

Average temperature during the infection periods ranged from 12.1ºC to 24.9ºC and, based on the lack of statistical significance, apparently it was not a limiting factor for infection during the periods of study. Previous reports from Korea indicated a temperature range for infection of 16.8-24.2ºC (Kang et al., 1993), somewhat narrow compared to that obtained in our study. Although infection efficiency of *M. nawae* ascospores at different temperatures has not been determined experimentally, our results suggest that this species can infect at relatively low temperatures, as was indicated in recent simulation studies (Makowski et al., 2011).

Measurable amounts of rain were recorded in 22 out of 26 weekly infection periods. However, rainfall and rain days were not correlated with disease incidence, probably due to the presence of some rain outside the periods of infection and inoculum availability. Cumulative rainfall during infection periods was 125 mm and 134.8 mm in 2010 and 2011, respectively. Even though these values were above normal, they were notably lower than the 300 mm recorded during infection periods in Korea (Kang et al., 1993).

Disease incidences of 1.8-21.9% were obtained in four weekly periods without measurable rain, and 10-69.6% in four periods with only one rain day and less than 1 mm precipitation. In contrast to the field studies from Korea, where infections were associated with high rainfall, our results suggest that light rains or even leaf wetness would be enough for *M. nawae* ascospores to germinate and infect. In fact, germination rates above 20% were obtained under laboratory conditions at 20ºC and 8 h of wetness, increasing to more than 80% with 15 h (Kwon et al., 1998c).

Inoculum in the leaf litter and airborne ascospores were correlated positively with disease incidence in both years, highlighting the potential of the ascospore discharge technique in disease forecasting. The relatively low proportion of ascospores in June was, however, able to

induce disease incidences up to 71.6% and 65% in 2010 and 2011, respectively. Moreover, a disease incidence of 3.3% was observed in the period of 1-8 July 2011, when ascospore concentration in the air was probably below the limit of detection of the spore trap and no catches were recorded. Therefore, quantitative relationships between ascospore catches and disease incidence should be carefully interpreted, especially considering that the study was conducted during the first years of the epidemic with high inoculum pressures.

Relative humidity was correlated negatively with disease incidence in both years. Following the pattern of normal values (AEMET, 2011), infection periods in April and June coincided with lower levels of relative humidity. Expected relationships among environmental variables, such as a negative correlation of temperature and a positive correlation of leaf wetness with both rainfall and rain days, were also observed.

Disease progress was best fitted to the monomolecular model in both years. Similar values of initial disease and rate of increase were obtained, except for Benimodo in 2010 where disease progressed faster. Although the nature of the disease cycle cannot be inferred from the disease progress curve, the monomolecular model has been successfully used to describe numerous monocyclic diseases (Pfender, 1982; Madden et al., 2007). Since no conidia were observed, all infections are likely to be caused by ascospores released from the leaf litter. Therefore, under semi-arid conditions in Spain, circular leaf spot can be considered monocyclic within a growing season and polyetic during successive growing seasons.

The monomolecular growth curve has been also proposed as the best model to describe the progress of diseases with a variable incubation period as a function of the host phenology (Bergamin-Filho and Amorim, 2002). In this group of diseases, symptom expression is a function of the phenological stage of the plant organ and has little relation to the time of infection. Despite differences in weather conditions, phenology and infection periods, symptoms of circular leaf spot appeared 120-127 DAFF in all experiments, 88-94 days after the last infections were detected on trap plants. The synchrony in symptom expression, coupled

with the production of toxins by *M. nawae* (Sassa et al., 1989), allow us to hypothesize that critical host-pathogen interactions might be triggered by specific physiological processes as described in other pathosystems (Walters et al., 2008).

In summary, *M. nawae* managed to adapt to semi-arid conditions in Spain mainly by displacing the period of inoculum production to coincide with rains and susceptible host availability. It appears that temperature ranges in the study area were not limiting either for ascospore release or infection. Although both years were characterized by rainfall above the normal, precipitation was considerably lower than that reported in southern areas of Korea where the disease is endemic. Actually, results obtained on trap plants indicated that ascospores of *M. nawae* were able to disseminate and infect under relative dry conditions. The unforeseen epidemic development of circular leaf spot in a semi-arid area highlights the limitations of climate suitability analyses, especially when based only on disease distribution records without considering detailed epidemiological data.

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References

Agencia Estatal de Meteorología, AEMET (2011). Valores climatológicos normales 1971-2000. http://www.aemet.es, accessed on 14 December 2011.

Arnal, L., & Del Río, M. A. (2003). Removing astringency by carbon dioxide and nitrogen-enriched atmospheres in persimmon fruit cv. Rojo brillante. *Journal of Food Science*, *68*, 1516-1518.

Aylor, D. E. (1996). Comparison of the seasonal pattern of airborne *Venturia inaequalis* ascospores with the release potential of *V. inaequalis* ascospores from a source. *Phytopathology*, *86*, 769-776.

Badenes, M., Garcés, A., Romero, C., Romero, M., Clavé, J., Rovira, M., & Llacer, G. (2003). Genetic diversity of introduced and local Spanish persimmon cultivars revealed by RAPD markers. *Genetic Resources and Crop Evolution*, *50*, 579-585.

Berbegal, M., Armengol, J., & García-Jiménez, J. (2011). Evaluation of fungicides to control circular leaf spot of persimmon caused by *Mycosphaerella nawae*. *Crop Protection*, *30*, 1461-1468.

Berbegal, M., Pérez-Sierra, A., Armengol, J., Park, C. S., & García-Jiménez, J. (2010). First report of circular leaf spot of persimmon caused by *Mycosphaerella nawae* in Spain. *Plant Disease*, *94*, 374.

Bergamin-Filho, A., & Amorim, L. (2002). Diseases with a variable incubation period as a function of the host phenology. *Fitopatologia Brasileira*, *27*, 561-565. (In Portuguese, abstract in English).

Campbell, C. L. & Madden, L. V. (1990). *Introduction to plant disease epidemiology* (p. 532) New York, USA: John Wiley & Sons, Inc.

Food and Agriculture Organization of the United Nations, FAO (2009). Crop production database FAOSTAT. http://faostat.fao.org/default.aspx, accessed on 24 July 2011.

Gadoury, D. M., & MacHardy, W. E. (1986). Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology*, *76*, 112-118.

García-Carbonell, S., Yague, B., Bleiholder, H., Hack, H., Meier, U., & Agustí, M. (2002). Phenological growth stages of the persimmon tree (*Diospyros kaki*). *Annals of Applied Biology*, *141*, 73-76.

George, A. P., Nissen, R. J., & Collins, R. J. (1994). Effects of temperature and pollination on growth, flowering and fruit-set of the nonastringent persimmon cultivar Fuyu under controlled temperatures. *Journal of Horticultural Science*, *69*, 225-230.

Giordani, E. (2003). El caqui: diversificación varietal para un cultivo en desarrollo. *Comunitat Valenciana Agraria*, *22*, 22-38. (In Spanish).

Ikata, S., & Hitomi, T. (1929). Studies on circular leaf spot of persimmon caused by *Mycosphaerella nawae*. *Special Bulletin of the Okayama Prefecture Agricultural Experiment Station*, *33*, 1-36. (In Japanese).

Jackson, S. L., & Bayliss, K. L. (2011). Spore traps need improvement to fulfil plant biosecurity requirements. *Plant Pathology*, *60*, 801-810.

Kang, S. W., Kwon, J. H., Lee, Y. S., & Park, C. S. (1993). Effects of meteorological factors on perithecial formation and release of ascospores of *Mycosphaerella nawae* from the overwintered persimmon. *Rural Development Administration Journal of Agricultural Science*, *35*, 337-343. (In Korean, abstract in English).

Kim, J. H. (2007). *Comparative analysis on ascospore liberation models for circular leaf spot of sweet persimmon caused by Mycosphaerella nawae*. Seoul, Korea: Seoul National University, M.Sc. thesis.

Kollar, A. (1998). A simple method to forecast the ascospore discharge of *Venturia inaequalis*. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz-Journal of Plant Diseases and Protection*, *105*, 489-95.

Kwon, J. H., Kang, S. W., Chung, B. K., & Park, C. S. (1995). Environmental factors affecting ascospore release of *Mycosphaerella nawae*, the causal organism of the spotted

leaf casting of persimmon. *Korean Journal of Plant Pathology*, *11*, 344-347. (In Korean, abstract in English).

Kwon, J. H., Kang, S. W., Cho, D. J., Shin, W. K., Park, C. S., & Kim, H. K. (1997a). Factor of affecting ascospore release of *Mycosphaerella nawae* causing spotted leaf casting of persimmon. *Rural Development Administration Journal of Crop Protection*, *39*, 10-14. (In Korean, abstract in English).

Kwon, J. H., Kang, S. W., Chung, B. K., Kim, D. K., Park, C. S., & Kim, H. K. (1997b). Improved chemical control measure of spotted leaf casting of persimmon caused by *Mycosphaerella nawae*. *Rural Development Administration Journal of Crop Protection*, *39*, 15-18. (In Korean, abstract in English).

Kwon, J. H., Kang, S. W., Park, C. S., & Kim, H. K. (1998a). Identification of the imperfect stage of *Mycosphaerella nawae* causing circular leaf spot of persimmon in Korea. *Korean Journal of Plant Pathology*, *14*, 397-401.

Kwon, J. H., Kang, S. W., Park, C. S., & Kim, H. K. (1998b). Microscopic observation of the pseudothecial development of *Mycosphaerella nawae* on persimmon leaves infected by ascospore and conidia. *Korean Journal of Plant Pathology*, *14*, 408-412.

Kwon, J. H., Kang, S. W., Park, C. S., & Kim, H. K. (1998c). Difference in temperature profiles optimum for germination of either ascospore and/or conidia, signifies the release as primary inoculum or secondary inoculum, respectively. *Rural Development Administration Journal of Crop Protection*, *40*, 80-82. (In Korean, abstract in English).

Kwon, J. H., & Park, C. S. (2004). Ecology of disease outbreak of circular leaf spot of persimmon and inoculum dynamics of *Mycosphaerella nawae*. *Research in Plant Disease*, *10*, 209-216. (In Korean, abstract in English).

Luley, C. J., & McNabb, H. S. (1989). Ascospore production, release, germination, and infection of *Populus* by *Mycosphaerella populorum*. *Phytopathology*, *79*, 1013-1018.

Madden, L. V., Hughes, G., & van den Bosch, F. (2007). *The study of plant disease epidemics* (p. 421) St. Paul, USA: APS Press, Inc.

Makowski, D., Bancal, R., & Vicent, A. (2011). Estimation of wetness duration requirements of foliar fungal pathogens with uncertain data. An application to *Mycosphaerella nawae*. *Phytopathology*, *11*, 1346-1354.

Pfender, W. F. (1982). Monocyclic and polycyclic root diseases. Distinguishing between the nature of the disease cycle and the shape of the disease progress curve. *Phytopathology*, *72*, 31-32.

Sassa, T., Igarashi, M., & Nukina, M. (1989). (-)-Mycousunine and (+)-isomycousunine, new phytotoxic usunic acid derivatives from the phytopathogenic fungus, *Mycosphaerella nawae*. *Agricultural and Biological Chemistry*, *53*, 1743-1744.

Vicent, A. (2008). Informe sobre los ensayos de tratamientos fungicidas en kaki. *Internal Report from Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia*. (In Spanish).

Vicent, A., Bassimba, D. D. M., & Intrigliolo, D. (2011). Effects of temperature, water regime and irrigation system on the release of ascospores of *Mycosphaerella nawae*, causal agent of circular leaf spot of persimmon. *Plant Pathology*, *60*, 890-908.

Walters, D. R., Havis, N. D., & Oxley, S. J. P. (2008). *Ramularia collo-cygni*: the biology of an emerging pathogen of barley. *FEMS Microbiology Letters*, *279*, 1-7.

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR Protocols, A guide to methods and applications (pp. 315–322). San Diego: Academic Press.

Whiteside, J. O. (1973). The possibilities of using ground sprays to control citrus greasy spot. *Proceedings of the Florida State Horticultural Society*, *86*, 19-23.

World Meteorological Organization, WMO (2011). World weather information service, monthly averages for the 30-year period 1971-2000. http://worldweather.wmo.int/index.htm, accessed on 14 December 2011.

Table 1 Pearson's correlation coefficient between incidence of circular leaf spot in trap plants, potential and airborne inoculum, and weather variables in 2010 and 2011.

Year/variables[a]	Incidence	Relative humidity	Rain days	Rainfall	Leaf wetness	Temp.	Airborne inoculum			
							Benimodo	L'Alcudia	Guadassuar	Castelló
2010										
Inoculum potential	0.6941**	-0.4879**	0.1355	0.0673	0.1127	-0.3098	0.7379**	0.6935**	0.7890**	0.5130**
Airborne inoculum	0.4249*	-0.2607	0.1550	0.1497	0.2657	-0.3198				
Temperature	-0.2154	0.0865	-0.4010*	-0.3639*	-0.1800					
Leaf wetness	-0.1525	0.5853**	0.3843*	0.4552*						
Rainfall	0.0375	0.3078	0.8300**							
Rain days	0.2733	0.1778								
Relative humidity	-0.5322**									
2011										
Inoculum potential	0.3972*	-0.2788	0.0306	0.0577	0.2324	0.0621	0.0773	0.4057*	0.1920	0.1606
Airborne inoculum	0.5668**	-0.2457	0.2247	0.4505*	0.0632	-0.1318				
Temperature	-0.2016	0.3166	-0.6943**	-0.5319**	-0.4911**					
Leaf wetness	0.2415	0.1253	0.3624*	0.4908**						
Rainfall	0.2142	0.1460	0.7541**							
Rain days	0.2586	-0.0459								
Relative humidity	-0.3684*									

[a] Thirty weekly periods from March to September in each year. Average value for temperature (ºC), leaf wetness (h) and relative humidity (%). Accumulated value for rainfall (mm) and rain days (>1 mm). **Significant at $P < 0.01$; *significant at $P < 0.05$.

Table 2 Parameter values and model fit summary statistics for the monomolecular disease progress curves of circular leaf spot in Benimodo and L'Alcúdia orchards in 2010 and 2011.

Year/orchard	Initial disease increase (y_o)	Rate of disease increase (r)	*RMSE*	*Pseudo-R^2*	*P*
2010 / Benimodo	0.2024 (0.1393 0.2665)[a]	0.2652 (0.2186 0.3118)	0.0271	0.9992	<0.0001
2010 / L'Alcudia	0.1974 (0.1116 0.2833)	0.1171 (0.0911 0.1430)	0.0392	0.9981	<0.0001
2011 / Benimodo	0.1140 (-0.0487 0.2767)	0.0855 (0.0565 0.1144)	0.0612	0.9955	<0.0001
2011 / L'Alcudia	0.1319 (-0.0131 0.2769)	0.0887 (0.0613 0.1161)	0.0543	0.9965	<0.0001

[a] Confidence intervals 95%.

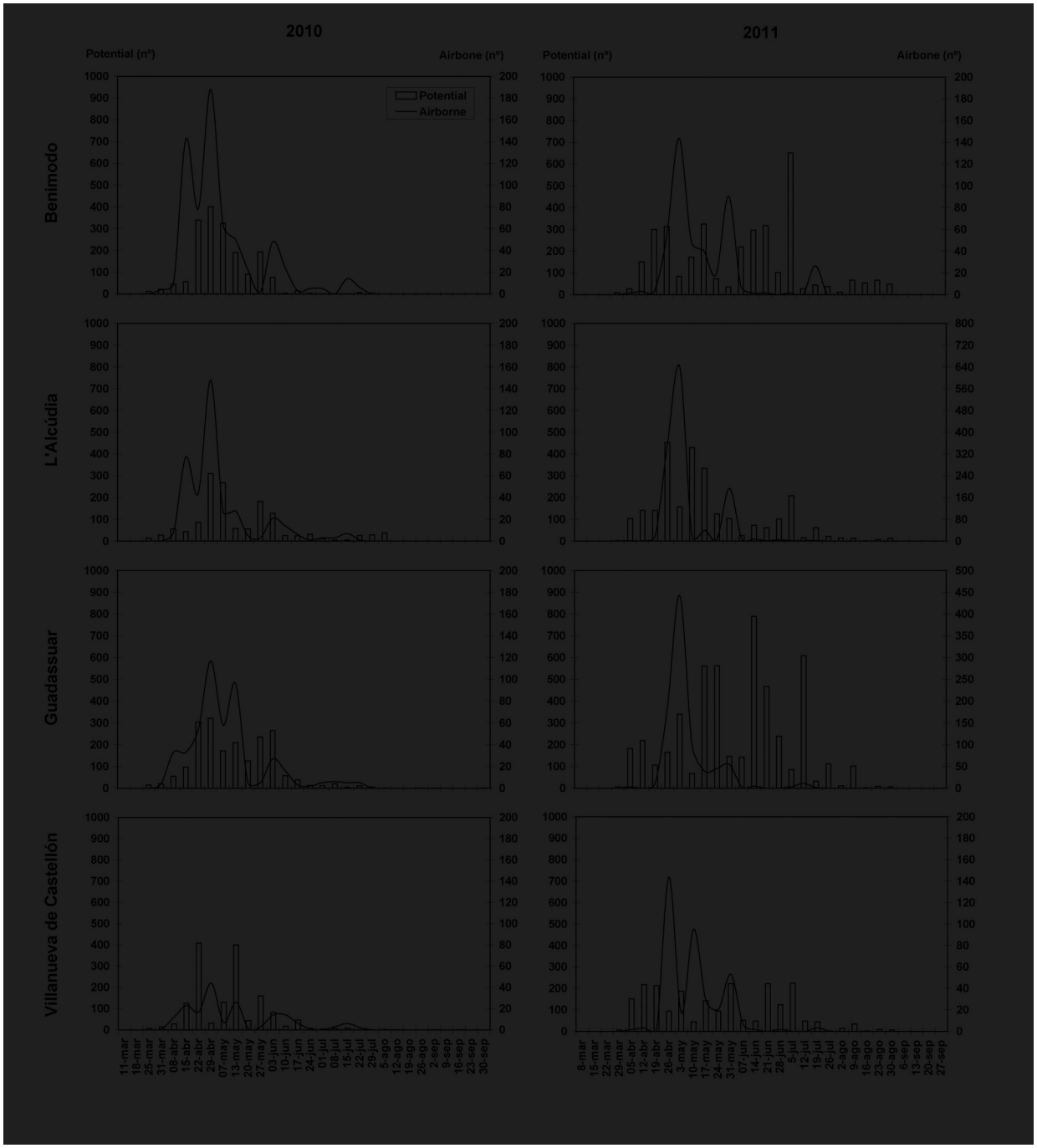

Fig. 1 Dynamics of ascospore potential in the leaf litter and airborne ascospores in four persimmon orchards affected by circular leaf spot in Valencia Province, Spain, from March to September in 2010 and 2011. Dates of the last day for each period.

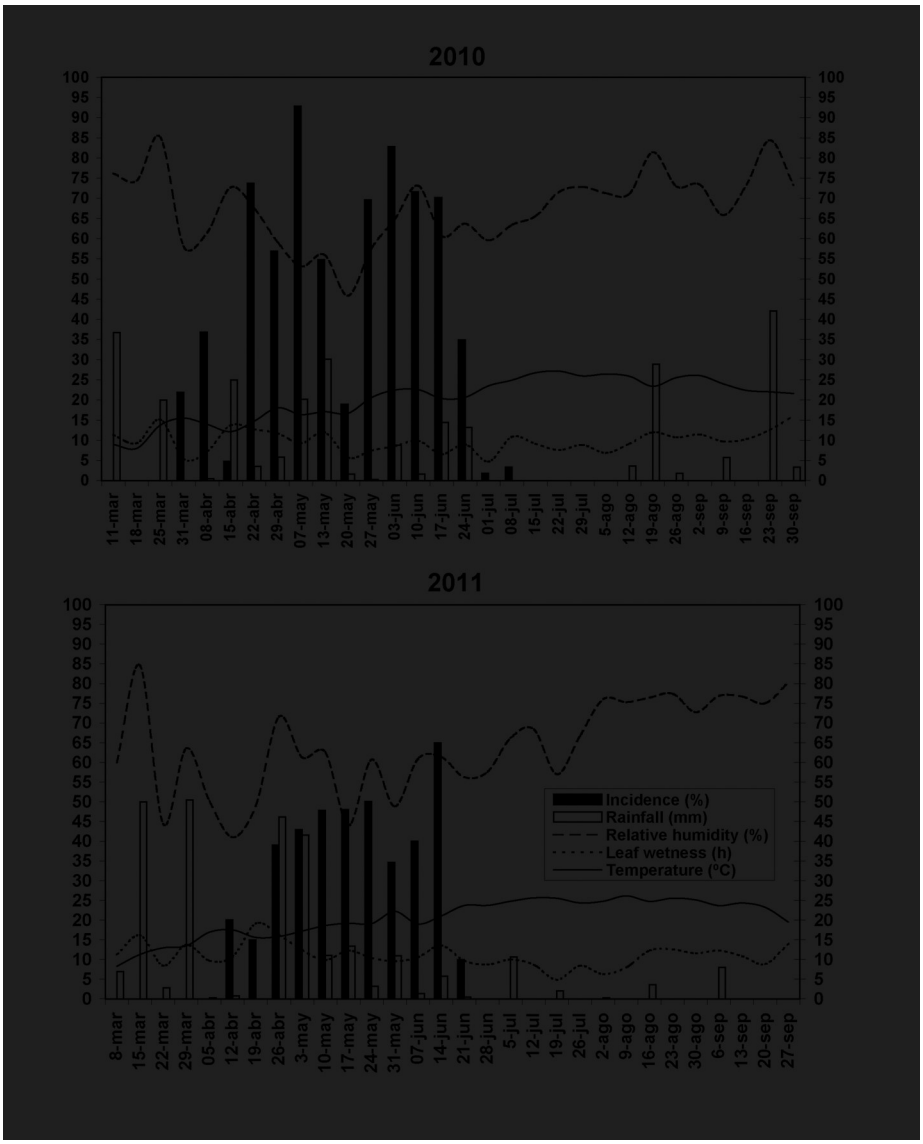

Fig. 2 Incidence of circular leaf spot on trap plants and environmental variables in Benimodo orchard from March to September in 2010 and 2011. Dates of the last day for each period.

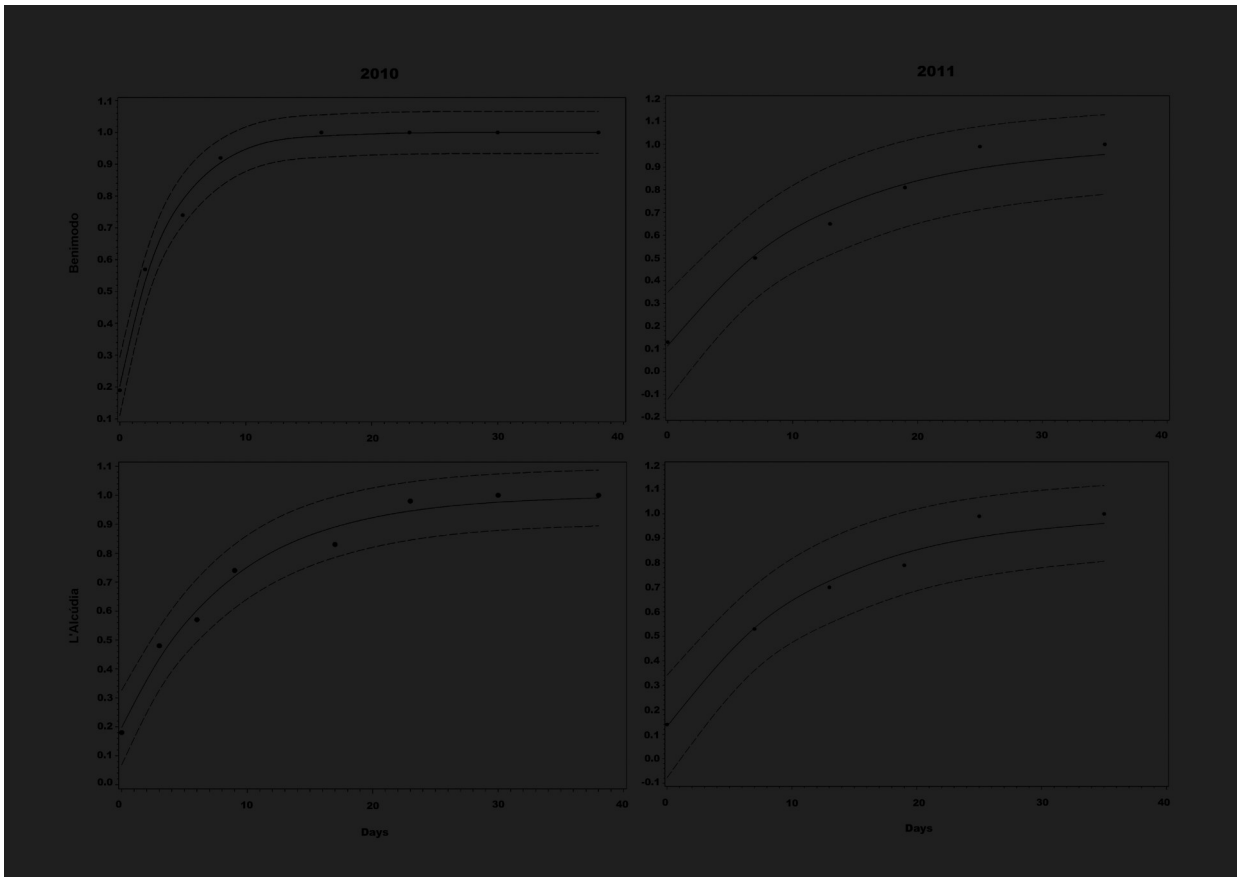

Fig. 3 Monomolecular disease progress curves of circular leaf spot incidence against days after disease onset in Benimodo and L'Alcúdia orchards in 2010 and 2011. Dots are the data obtained in the experiments, the solid line shows the regression model fit to the data, and the dashed lines are the 95% confidence levels for the response.