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3**Effects of Temperature, Water Regime and Irrigation System on the Release of**

4**Ascospores of *Mycosphaerella nawae*, Causal Agent of Circular Leaf Spot of Persimmon**

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18Abstract

19 The fungal pathogen *Mycosphaerella nawae*, which causes circular leaf spot of persimmon,
20was recently found causing epidemics in Spain. Prior to this detection, the disease was confined
21to areas of Korea and Japan characterized by a summer-rainfall pattern and high precipitation.
22In contrast, persimmon-growing areas in Spain are semi-arid and orchards are irrigated. Most of
23the information about the dynamics of *M. nawae* ascospore production has been derived from
24field studies conducted in Korea, which cannot be easily extrapolated to other geographical
25areas. The aim of this study was to quantify the effects of temperature, water regime, and
26irrigation system on the release of *M. nawae* ascospores from leaf litter. The effect of
27temperature on ascospore release was best described by a Gompertz model. The end of the lag

phase of ascospore release occurred at 9.75ºC, and the end of the exponential phase at 15.75ºC. Few ascospores were discharged from dry leaves wetted with 0.1 and 0.5 mm of water, but significant amounts were recovered with 1 to 50 mm water. About half of the total ascospores were released after three wetting and drying cycles, but 32 cycles were necessary for a complete discharge. No significant difference in ascospore release was detected when the leaf litter was wetted by flood and drip irrigation. However, considering the proportion of soil area wetted in both systems, inoculum release was significantly reduced by drip irrigation. The potential of drip irrigation as a cultural control measure should be investigated.

Keywords: Diospyros kaki, epidemiology, dew, rainfall, surface irrigation

Introduction

Persimmon (*Diospyros kaki*) is a deciduous fruit tree crop widely grown in areas of Far East Asia such as China, Korea and Japan, and it is also locally important in Brazil, Azerbaijan, Italy and Israel. In east central Spain, the persimmon-growing area has increased considerably due to the popularisation of the cultivar 'Rojo brillante' and the implementation of the postharvest deastringency treatment, which opened new export markets.

Circular leaf spot disease of persimmon, caused by *Mycosphaerella nawae*, was first described in Japan (Ikata & Hitomi, 1929), and is prevalent in Korea (Kang *et a*l., 1993). Both areas have a humid-subtropical-type climate, with a characteristic summer-rainfall pattern and an annual precipitation > 1,500 mm which allows growing persimmon without irrigation. Until its detection in Spain, the disease was confined to this particular eco-climatic region.

In 2008, a leaf spot disease of persimmon was detected in Valencia province in east central Spain. The disease induced leaf necrosis, premature defoliation and early maturation and fruit abscission, resulting in severe economic losses in 2008 and 2009. The first insight into the aetiology of the disease was obtained from a field trial for the control of *Botrytis cinerea*.

Fungicide-treated trees had a significantly lower leaf spot intensity compared to the nontreated ones (Vicent, 2008). Finally, Koch postulates were completed and the causal agent of the disease was identified as *M. nawae* (Berbegal *et a*l., 2010).

M. nawae overwinters as pseudothecia in leaf litter. Once mature, ascospores are forcibly discharged from pseudothecia when specific temperature and moisture requirements are met. Ascospores are disseminated long distances by air currents, infecting persimmon leaves in the presence of a film of water and adequate temperatures (Kwon & Park, 2004). In Korea, secondary inoculum consisting of *Ramularia*-type conidia has been described, but it is considered of minor epidemiological importance compared to ascospores (Kwon & Park, 2004). The asexual stage has not been reported in Spain.

In contrast to Korea and Japan, persimmon-growing regions in Spain are characterized by a semi-arid Mediterranean-type climate. Annual precipitation is about 500 mm, distributed during the early spring and fall periods, with a characteristic rainless summer. Because of this, most of the persimmon orchards are surface (flood) irrigated. This type of irrigation system consists of wetting the entire orchard floor with large water volume (50 to 100 mm) applied with very low frequency, e.g. 1-2 times per month. As in other fruit tree crops in Spain, new programs have been initiated to convert persimmon orchards to drip irrigation. This system only wets a small portion of the orchard floor (5 to 10%) with a rate of 2-3 mm, although the frequency of the irrigation events is very high.

Together with the presence of a susceptible host and favourable environmental conditions over a sufficient period, infection is determined by the availability of inoculum. The presence of the pathogen is particularly critical in the case of monocyclic foliar pathogens, whose spores are present only during a limited period of time. Most information on the dynamics of *M. nawae* inoculum has been derived from the epidemiological research conducted in Korea. These studies indicated that the most important factors affecting the presence of airborne ascospores were rain and an average temperature > 15ºC (Kang *et a*l., 1993; Kwon *et a*l., 1995; 1997).

These studies were essentially empirical, developed from field observations of ascospore counts and weather data, and analysed in a descriptive way. Although extremely valuable, conclusions obtained from these experiments are limited to the area of study and cannot be extrapolated to other geographical regions, especially considering the different climatic conditions and cropping systems in Korea.

Field studies integrate much of the complexity involved in inoculum dynamics at the population level. The relationship between weather variables and the presence of inoculum can be derived from long-term field experiments. However, in these kinds of experiments, it is difficult to differentiate the effects of the environment on pseudothecial maturation from those on ascospore release. To understand the underlying biological processes, field studies should be combined with experiments under controlled conditions, where each component of the inoculum production process can be studied separately. In addition, the range of environmental factors that can be evaluated in controlled experiments is much wider than the range likely to occur under field conditions, so results are not constrained to the area of study.

The objective of this study was to quantify the effects of temperature, water regime and irrigation system on the release of *M. nawae* ascospores under controlled conditions. This study will complement the epidemiological field experiments currently in progress. Factors involved in inoculum release are key components to assess the risk of adaptation of *M. nawae* to new environments as well as to design more efficient disease control strategies.

Materials and methods

Effect of temperature

Dry leaves bearing mature pseudothecia of *M. nawae* were collected in 2010 from the soil of an affected orchard located at Benimodo in Valencia Province, Spain (39ºN, elevation 42 m).

Pseudothecial maturity was assessed by squashing 50 pseudothecia on microscope slides in lactophenol-acid cotton blue. Mountings were examined at 400X and the maturity of each pseudothecium was rated on the 1-7 scale described by Trapero-Casas & Kaiser (1992): stage 1, stromatic pseudothecial initial; stage 2, pseudoparaphyses filling the lumen of the pseudothecium; stage 3, appearance of asci arising among pseudoparaphyses; stage 4, asci formed but contents not differentiated; stage 5, asci with ascospores being formed or completely formed and mature, very few pseudoparaphyses remaining; stage 6, empty or half-empty asci and released ascospores; stage 7, empty pseudothecium, all ascospores have been discharged and some asci walls can be detected. These leaves were used in all the experiments.

A preliminary test was conducted to determine the optimum duration of ascospore collection to be used in further experiments. Two samples of 10 dry leaves (≈ 7 g per sample) were used for each temperature evaluated (5, 10, 15 and 20ºC). To collect ascospores, dry leaves were soaked for 15 min in distilled water and placed with the abaxial surface facing upward in a wind tunnel (Whiteside, 1973). The device consisted of a plastic/aluminium tray 640 mm long, 300 mm wide, and 60 mm deep. One end had two tubes through which air was introduced by a pump. The other end was tapered to a vent (9 mm diameter) to which a glass microscope slide (26 x 76 mm) coated with silicone oil (Merck) was attached at 20 mm. Wind speed at the vent was adjusted to ≈ 3.5 m s^{-1}. Microscope slides were treated with lactophenol-acid cotton blue and a coverslip (22 x 22 mm) was affixed. Slides were examined at 400X magnification and all *Mycosphaerella* ascospores were counted in four microscope field transects. In this preliminary test, ascospores were collected for 30, 60, 90 and 120 min. The experiments were carried out in a temperature-regulated chamber (Hotcold L, Selecta). Water for leaf soaking was maintained at the same temperature. In all cases, temperature was monitored with ± 0.2ºC accuracy using a sensor connected to a datalogger (Hobo TMCx-HD, Onset Computer Corporation).

Two experiments were conducted using three samples of 10 leaves (≈ 7g per sample) for each temperature. In both experiments, temperature sequences were assigned randomly and ascospores were collected for 40 min. The temperatures tested in the experiments were 5, 10, 15, and 20ºC, and 9, 10, 11, and 12ºC respectively. The average ascospore counts at each temperature were calculated and analysed using non linear models with the NLIN procedure of SAS 9.0 (SAS Institute Inc.).

Effect of water regime

To evaluate the effect of different quantities of water on ascospore release, dry leaves were placed with the abaxial surface facing upward sandwiched between two plastic mesh frames with 5-by-5-mm openings. Frames were placed on soil in plastic containers (270 mm long, 175 mm wide, 55 mm deep) prior to the water treatments. Plastic containers had five orifices (16 mm diameter) at the bottom to drain the excess of water. The soil was obtained from an orchard located at Moncada in Valencia Province, Spain, and was a sandy loam to sandy clay loam texture with an available water capacity of 0.125 m m^{-1}. More details about soil characteristics can be obtained from Gonzalez-Altozano & Castel (1999). Water was applied to the surface of the leaves with a manual pressure sprayer. The water volumes tested were 0, 0.1, 0.5, 1, 5, 10, and 50 mm. Three replicates of 10 dry leaves (≈ 7g) were used for each water amount. Air and water temperature was maintained at ≈ 21°C. After 15 min, leaves were placed in the wind tunnel for 40 min. Ascospores were collected and counted as described above. Data were analysed with generalized linear models with a log link function using the GENMOD procedure of SAS 9.0. Overdispersion was detected in this data, so a negative binomial distribution was used instead of the poisson. Contrasts between treatments were performed with a χ^2 test (Schabenberger & Pierce, 2001).

Potential infectious period

The potential infectious period of *M. nawae* was determined by alternate wetting and drying of dry leaves. Four samples of 10 dry leaves each (≈ 7 g) were soaked for 15 min in distilled water and placed in the wind tunnel for 45 min. Ascospores were collected and counted as described above. Then, leaves were dried under laboratory conditions and the process was repeated on a daily basis for 32 days. Air and water temperature was maintained at ≈ 21°C. Data were analysed by calculating mean ascospore counts and standard errors.

Effect of irrigation system

The effect of flood and drip irrigation on ascospore release was determined in small-scale enclosed experiments, and under field conditions. Experiments were conducted at a site in Moncada, Spain at the IVIA research station (39ºN, elevation 69 m), which had soil with the same characteristics as already described. Weather conditions were recorded with an automatic meteorological station (AWS, Campbell Scientific) located at the experimental site. Leaf litter density in the experiments was adjusted to ≈ 350g of dry leaves m^{-2}, the density usually present in persimmon orchards.

The treatments evaluated were flood irrigation, drip irrigation, and a non-irrigated control. Flood irrigation consisted on wetting the entire experimental area with 70 mm water applied over a period of 65 min. Drip irrigation was applied with pressure compensated emitters (PC, Netafim) with a discharge rate of 4 L h^{-1}, with water application lasting 2 hours. The soil area wetted by each emitter was photographed at 300 ppi using a digital camera (Coolpix 4500, Nikon Corporation) using a ruler for measurement of scale. The soil area wetted was quantified using Assess V2.0 (American Phytopathological Society).

A plastic spore-trap modified from that described by Holb (2006) was used for the experiments under enclosed conditions. In each experimental unit of 6 x 6 m, 25 g of dry leaves were placed in a 0.30 x 0.24 m sampling area. Leaves were placed on the soil with the abaxial surface facing upward and were covered with a plastic mesh (5-by-5-mm openings) fixed with four stainless steel pins. Four glass microscope slides coated with silicone oil were placed arbitrarily above the plastic mesh (≈ 5 mm above the leaves) facing downward, and the sampling area was covered by a translucent plastic container (300 mm long, 240 mm wide, 140 mm deep). Treatments were replicated four times in a completely randomised design. In the drip irrigation treatment, emitters were placed below the leaves in the centre of the sampling area. In the whole experimental unit area, a total of six emitters were placed in two irrigation lines (3 emitters per line). The coated glass slides were exposed for nine hours (10:00 - 19:00) after the onset of irrigation. When the glass slides were removed, leaves were completely dry. Microscope slides were stained with lactophenol-acid cotton blue and a coverslip (24 x 32 mm) was affixed. Slides were examined at 400X magnification and all *Mycosphaerella* ascospores were counted in three microscope field transects. The experiment was repeated once.

For the open-field experiments, 1,300 g of dry leaves were placed on the surface of lysimeters with an area of 3.78 m^2 and 1 m height. The lysimeters were located in a rectangular grid with a separation between them of 1.25 m in both directions. The flood irrigation treatment was performed as described above. For the drip irrigation treatment, a single emitter was located in the centre of the lysimeter. Two glass slides coated with silicone oil were placed facing downward in the centre of each lysimeter with a deployment angle of 45º (≈ 50 mm above the leaves). Treatments were replicated four times in a completely randomised design using one lysimeter per replication. Glass slides were exposed for nine hours and ascospores were counted as described above. The experiment was repeated once. Soil water content was determined in all lysimeters gravimetrically in the 0-0.3 m soil depth by measuring the water content of soil cores extracted 5 hours after irrigation. Six cores were taken in each of two

transects to ensure that the soil water content measurements were representative of the average soil water content for the whole lysimeter.

Data were analysed with generalized linear models using the GENMOD procedure of SAS 9.0. The negative binomial distribution was used to compensate for the problem of overdispersion. Contrasts between treatments were performed with a χ^2 test (Schabenberger & Pierce, 2001). In the experiments with enclosed spore traps, results were scaled from the sampling area to the experimental unit to estimate the population totals and their associated standard errors using the formulas indicated by Thompson (2002):

$$\tau = N\,y \qquad (1)$$

$$SE = [N^2\,var(y)]^{1/2} \qquad (2)$$

where τ = estimate of population total, N = population size, y = sample mean, and $var(y)$ = variance of the sample mean.

Results

Effect of temperature

The calculated maturity index of the pseudothecia was 5.88, corresponding to 12% of immature pseudothecia (categories 3 and 4), 50% of mature pseudothecia (categories 5 and 6), and 38% of empty pseudothecia (category 7). In the preliminary test, no ascospores were detected in the treatment of 5ºC. At 10ºC, only seven ascospores were detected in total. For 15ºC and 20ºC, the sum of ascospores detected in both samples was 146 and 160 respectively. At 15ºC, the average cumulative percentage of ascospore recovery in 60 min was 77%. This percentage was higher than 95% at 20ºC.

The relationship between temperature and ascospore release (Fig. 1) was best described by the Gompertz model (Winsor, 1932):

$$asc = a \exp [-\exp (b - cT)] \quad (3)$$

where *asc* = number of ascospores at temperature *T*, and *a*, *b, c* = model parameters. The estimated parameter values were: a = 301.3 ± 52.78 SE, b = 4.08 ± 1.29 SE, and c = 0.32 ± 0.12 SE ($P = 0.0003$, pseudo-R^2=0.96, RMSE = 28.38). According to the formulas indicated by Zwietering *et a*l. (1992), the end of the lag phase of ascospore release was calculated at 9.75ºC, and the end of the exponential phase at 15.75ºC, with an inflection point at 12.75ºC.

Effect of water regime

An average of five and 16.5 ascospores were detected with 0.1 and 0.5 mm water respectively (Fig. 2). The average number of ascospores detected with the treatments of 1, 5, 10 and 50 mm water ranged from 478 to 1,628.7. No ascospores were detected in the 0 mm water treatment; therefore, it was not included in the statistical analysis to avoid problems of heteroscedasticity. The ratio between the deviance and the degrees of freedom obtained with the negative binomial distribution was 1.6051. The factor treatment was significant ($P < 0.0001$). A significantly lower ($P < 0.05$) number of ascospores was detected with the treatment of 0.1 mm water compared to 0.5 mm water. The number of ascospores recovered from the treatments with 0.1 and 0.5 mm water were significantly lower ($P < 0.05$) compared to the other water volumes evaluated. No significant differences were detected ($P > 0.05$) among the treatments of 1, 5, 10, and 50 mm.

Potential infectious period

Thirty-two cycles of wetting and drying were required to exhaust the ascospores in the samples of leaf litter evaluated (Fig. 3). An average cumulative percentage of ascospore recovery of

151% was obtained with three wetting and drying cycles. This percentage increased up to 75%
2after nine cycles, and to 90% after 18 cycles.
3
4**Effect of irrigation system**
5
6The average environmental conditions during the experiments with enclosed spore traps were:
7air temperature 28.8ºC, relative humidity 38.2%, and wind speed 2 m s^{-1}. The average
8conditions during the open-field experiments were: air temperature 29.2ºC, relative humidity
962.6%, and wind speed 2.1 m s^{-1}. No rain was recorded during the experiments.
10 No ascospores were detected in the non-irrigated controls of the experiments with enclosed
11spore traps (Fig. 4). Average ascospore counts ranged in the flood irrigation treatment from
121.75 to 6.25 and from 0.67 to 1.67 in the drip irrigation treatment. The ratio between the
13deviance and the degrees of freedom obtained with the negative binomial distribution was
141.3034. Neither of the two factors studied and their interaction was statistically significant:
15'experiment' (P = 0.2712), 'treatment' (P = 0.2590), and 'experiment x treatment' (P =
160.8524). Results were scaled from the sampling area to the experimental unit considering that:
17no ascospores were detected in the non-irrigated control; and the area wetted in the flood and
18drip irrigation was 1.50 and 36 m^2 respectively. The estimated number of ascospores in the
19experimental unit was 24.57 ± 18.30 SE for drip irrigation and 2,000 ± 1,388.34 SE for flood
20irrigation.
21 Average soil water content in the open-field experiments was 21.5% for the flood irrigation
22treatment, 11.5% for the drip irrigation treatment, and 8.5% for the non-irrigated control. The
23average soil area wetted in the lysimeters was 18% in the drip irrigation treatment and 100% in
24the flood irrigation treatment. Average ascospore counts ranged from 3.25 to 8 in the flood
25irrigation treatment, from 0 to 1 in the drip irrigation treatment, and 0.5 in the non-irrigated
26control. The ratio between the deviance and the degrees of freedom obtained with the negative

binomial distribution was 1.2338. The factors 'experiment' ($P = 0.0074$) and 'treatment' ($P < 0.0001$) were statistically significant, but their interaction was not ($P = 0.1282$). Ascospore counts in the flood irrigation treatment was significantly higher compared to the drip irrigation treatment ($P < 0.0001$). It was not possible to estimate the contrasts including the non-irrigated control due to the high proportion of zero values in this treatment.

Discussion

Under non-limiting moisture conditions, the release of *M. nawae* ascospores began at temperatures around 10ºC and increased exponentially to nearly 16ºC. The release of ascospores of *Erysiphe necator*, *Venturia inaequalis* and *V. nashicola* was also reduced at temperatures below 10°C. However, in contrast to *M. nawae*, the effect of temperatures above 10°C was minimal for the above-mentioned species (Gadoury & Pearson, 1990; Stensvand *et al*., 1997; Lian *et al*., 2007). For *Monilinia fructicola*, the rate of ascospore release increased as temperature rose from 10 to 15°C (Hong & Michailides, 1998). The differences among these studies is most likely due to a combination of taxonomic and biological differences among species, and due to the methods used in experiments. Considering that the vegetative growth of persimmon takes place above 10ºC (George *et al*., 1994), temperature does not appear to be limiting for the release of mature ascospores when susceptible host tissues are available.

The majority of ascospores were released within 60 min after soaking the leaves. This result is in agreement with previous studies conducted in Korea, where up to 92% of the total ascospores were released within one hour after soaking (Kwon *et al*., 1995; 1997). Similar results were described for other ascomycetes (Gadoury *et al*., 1996; Mondal *et al*., 2003). In the present study, leaves carrying 62% mature and immature pseudothecia released about half of the total ascopores after three soakings. This is consistent with field studies conducted in Korea (Kang *et al*., 1993), where *M. nawae* ascospores began to be trapped after the first day of rain

and catches decreased sharply after three days of precipitation. In the case of *M. citri*, all pseudothecia had matured and released their ascospores after three to four cycles of wetting and drying of grapefruit leaves (Mondal *et a*l., 2003). However, in the present study, up to 31 wetting and drying events were necessary, indicating a declining source of inoculum with a potentially long infectious period due to the progressive maturation of pseudothecia.

The mechanism of forcible discharge in ascomycetous fungi is known to be driven by the influx of water and turgor pressure within the ascus (Trail, 2007). The effect of moisture on ascospore release has been extensively described for *M. nawae* and other *Mycosphaerella* species (Kang *et a*l., 1993; Kwon *et a*l., 1995; 1997; Burt *et a*l., 1999; Mondal *et a*l., 2003). In our study, no ascospores were released in the absence of water at non-limiting temperatures. Few ascospores were released from dry leaves on a soil surface sprayed with 0.1 and 0.5 mm of water, but significant numbers were recovered with 1 to 50 mm. The two lowest water rates simulated average daily dew rates and the others represented different amounts of rain (Moro *et a*l., 2007; Xiao *et a*l., 2009).

The effect of rain on the release of ascospores of *M. nawae* under field conditions was previously reported (Kang *et al*., 1993; Kwon *et al*., 1995). Controlled experiments evaluating different soaking periods were also published, but not comparing different water amounts (Kwon *et a*l., 1997). Although depending on soil and orchard characteristics, a rain event of 5 mm is considered sufficient to wet at least the top layer of the leaf litter (McOnie, 1964). However, our results indicated that 1 mm would be enough to release significant numbers of *M. nawae* ascospores. Higher water volumes evaluated did not increase the number of ascospore recovered from leaves significantly.

No studies are available on the effect of dew on the release of *M. nawae* ascospores. For other ascomycetes, measurable numbers of ascospores were detected during dew periods (McCoy & Dimock, 1973; Latorre *et a*l., 1985; Pusey, 1989; Stensvand *et a*l., 1998). However, other studies considered that dew was insufficient to allow a significant release of ascospores

(MacHardy & Gadoury, 1986; Rossi *et a*l., 2001; Alt & Kollar, 2010), and the differences between studies are likely to be due to species of pathogen and the trapping efficiency of the various spore samplers used in these studies.

Our results showed that low numbers of ascospores were recovered by spraying 0.1 and 0.5 mm of water. With this application method water was delivered in a few seconds. However, under natural conditions the rate of dew condensation is slower, and more time would be required to reach the moisture threshold for ascospore release (McCoy & Dimock, 1973, Stensvand *et a*l., 1998). The potential effect of raindrop impact on ascospore discharge should be also considered (Alt & Kollar, 2010). In any case, the epidemiological significance of ascospores that might be released during dew periods will depend on their infection efficiency and the overall inoculum level in the orchard.

In the experiments with enclosed spore traps, sampling areas in the flood and drip irrigation treatments were wetted with 70 and 32 mm water, respectively. These amounts are large enough for ascospore release (Fig. 2), consequently no significant differences were detected between the irrigated treatments. However, if data are scaled to the experimental unit considering the proportion of soil area wetted in each treatment, the number of *M. nawae* ascospores was dramatically reduced by drip irrigation.

In the open field experiments, although statistically significant, differences were not as noteworthy. The fact that some ascospores were detected in the non-irrigated lysimeters indicated that results were affected by interplot interference. Dispersal gradients of *M. nawae* ascospores should be determined in order to select an adequate plot size for future experiments. Passive spore traps used in the irrigation experiments showed lower capture efficiency than the wind tunnel of the laboratory experiments. Holb (2006) did not provide ascospore counts, but the collection efficiency of this spore trap would be improved by incorporating an air flow device (Sutton *et a*l., 2000). This would also reduce the considerable variability observed in the experiments (Fig. 4).

The effect of flood irrigation on ascospore release was described for *M. citri* (Timmer *et al*., 1980), but few studies are available comparing the effect of drip and flood irrigation on plant disease epidemics. Most studies evaluated the effects of drip and sprinkler irrigation, particularly on diseases caused by splash-dispersed pathogens (Palti, 1981). When compared with flood irrigation, sub-surface drip irrigation reduced the intensity of Alternaria late blight in pistachio orchards in California (Goldhamer *et al*., 2002). The buried drip system reduced soil surface evaporation, orchard humidity and dew duration.

In the case of the circular leaf spot of persimmon, the disease spread very fast in east central Spain causing severe epidemics in 2008 and 2009. Virtually all persimmon orchards in the affected area are flood irrigated, so this factor was thought to increase the rate of disease progression by favouring ascospore release and leaf wetness formation. Apart from improving the orchard water use efficiency, moving to drip irrigation might provide disease control by reducing exposure of the leaf litter on the soil surface to moisture and decreasing ambient humidity in the orchards. However, it should be validated in field studies considering the interactions among irrigation timing, host development, inoculum availability, and micro-mesoclimatic variables.

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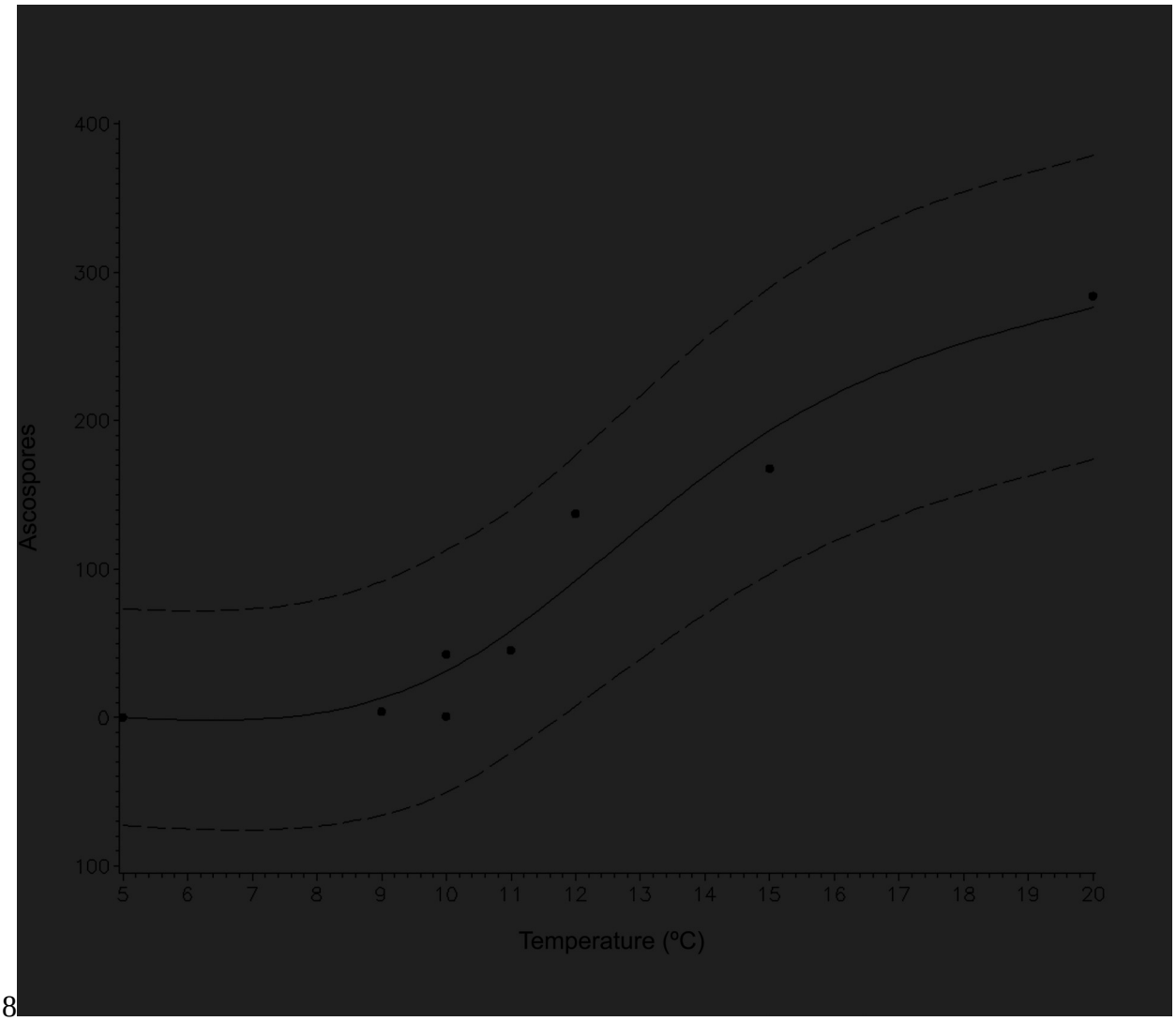

Figure 1: Effect of temperature on the release of ascospores of *Mycosphaerella nawae* under controlled conditions. Dots are the data obtained in the experiments, the solid line shows the regression model fit to the data, and the dashed lines are the 95% confidence levels for the response.

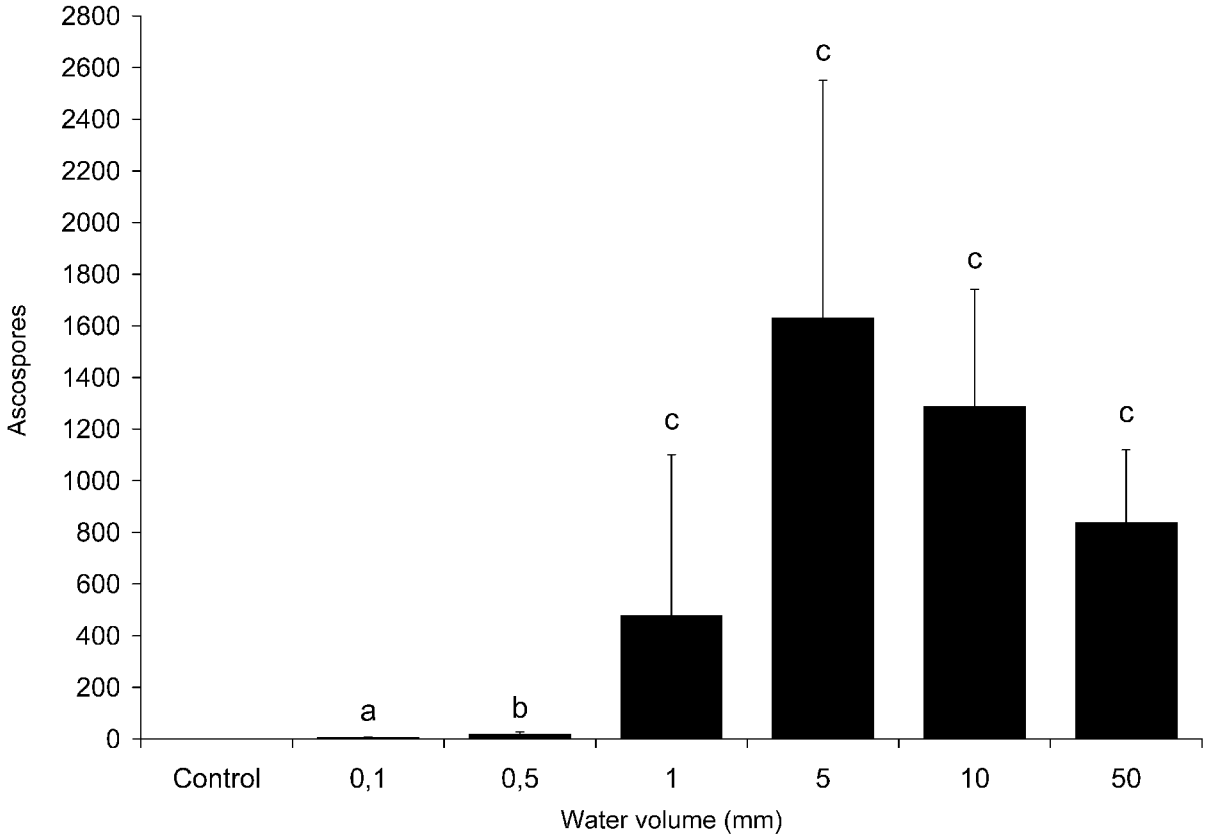

Figure 2: Effect of water volumes on the release of ascospores of *Mycosphaerella nawae* under controlled conditions. Numbers followed by the same letter are not significantly different (P > 0.05). Bars equal SE of the means.

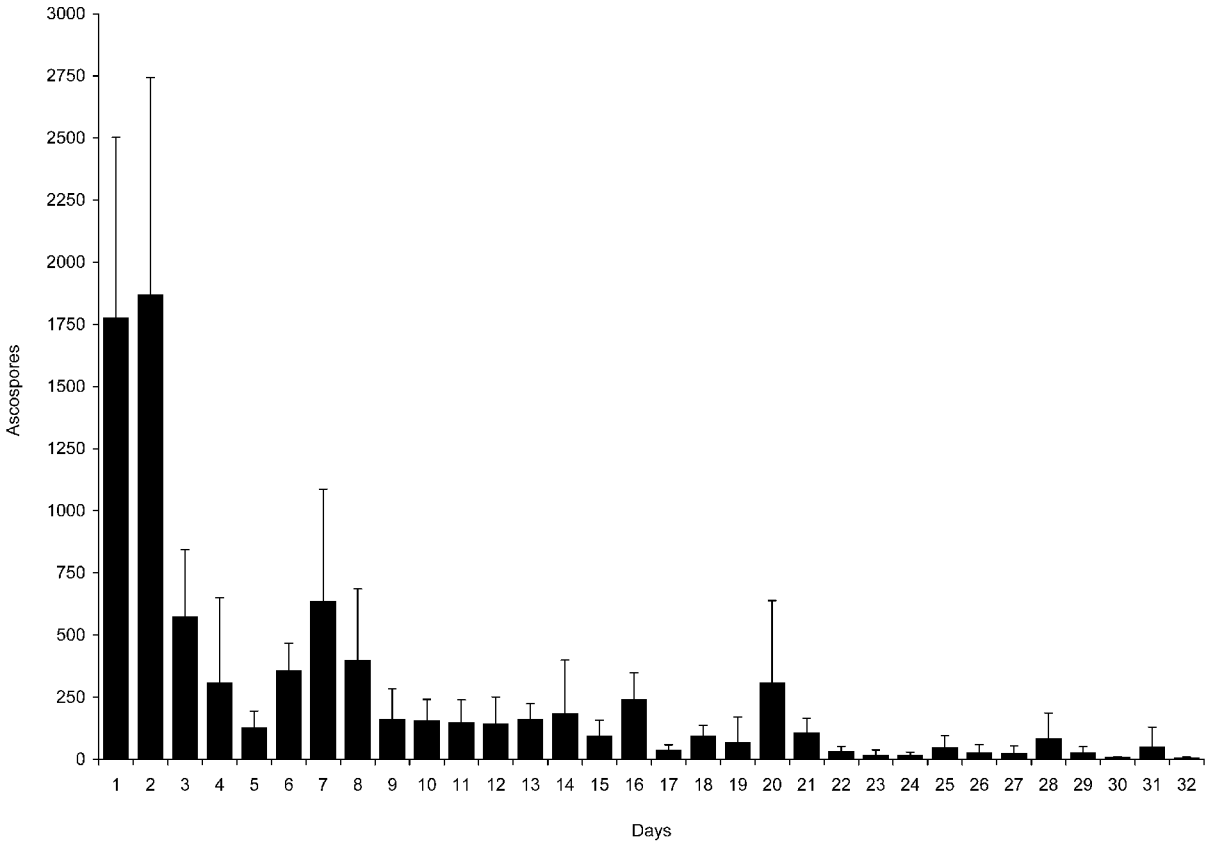

Figure 3: Effect of sequential leaf wetting and drying on the discharge of ascospores of *Mycosphaerella nawae* under controlled conditions. Bars equal SE of the means.

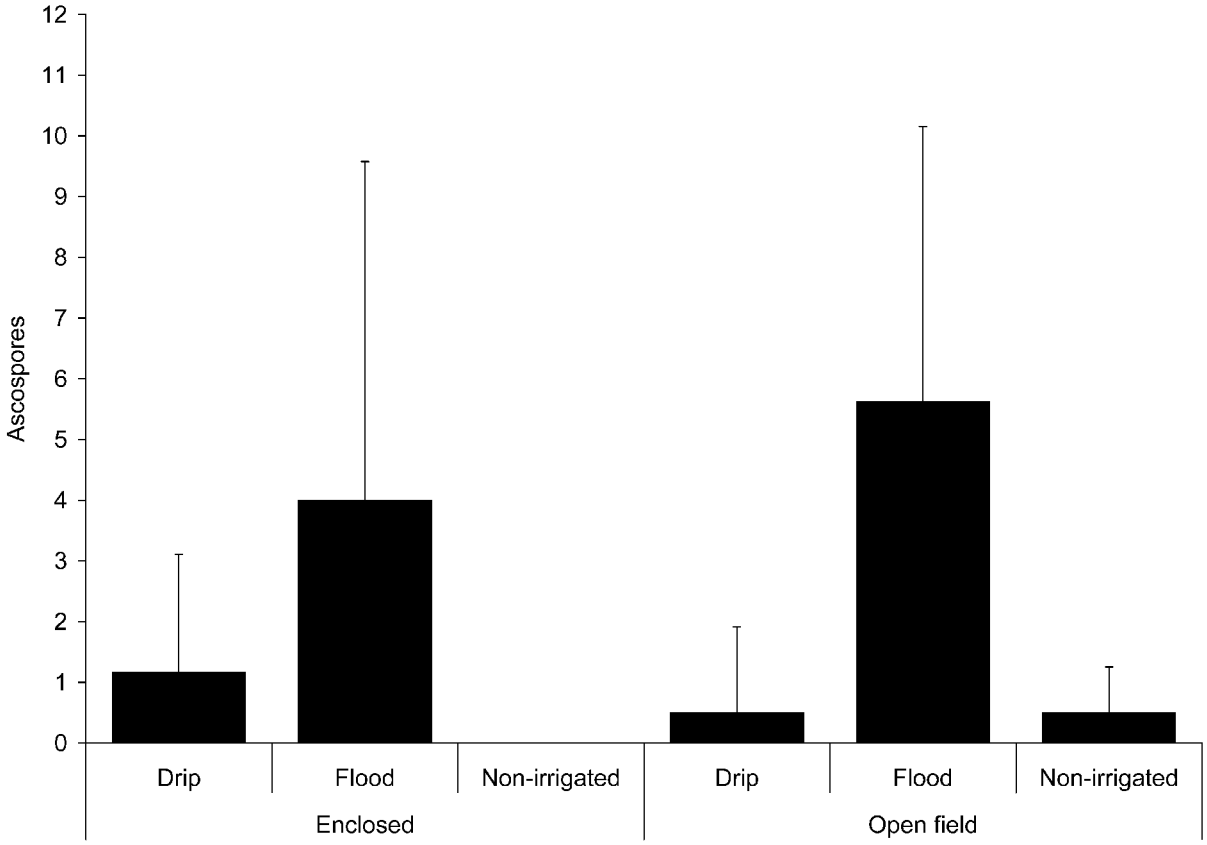

Figure 4: Effect of irrigation on the release of ascospores of *Mycosphaerella nawae* under enclosed and open-field conditions in an experimental site in Moncada, Spain, in 2010. Data from enclosed spore traps are not scaled considering the soil area wetted. Bars equal SE of the means of two experiments.