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An assay for the quantification of pathogenicity and virulence of two strains of *Podosphaera xanthii* (Erysiphaceae) on different hosts from digital images

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The predominant species of powdery mildew in the Cucurbitaceae is *Podosphaera xanthii*. However, there is little information on the epidemiology of this disease in Mexico despite its importance. As a first assay, we explore the applicability of a quantitative technique to estimate differences in the severity of disease caused by *P. xanthii* on leaves of Cucurbitaceae. We obtained two samples from different hosts: cultivated (*Cucurbita pepo*) and wild (*C. radicans*) cucurbits. Conidia were inoculated onto the leaves of growing plants of seven cucurbit species. We used the ImageJ program to quantify the infected area for each leaf as the sum of several polygons. The severity of the infection was calculated as the percentage of the infected foliar area. The two inocula of *P. xanthii* were pathogenic in five of seven inoculated cucurbits. Our analyses of variance to compare the variation of the percentage values among susceptible hosts revealed different levels of severity. This result indicates that this is a promising method to quantitatively compare differences in disease severity of different strains or hosts of powdery mildews.

Keywords: cucurbits, image analysis, infection percentage, powdery mildew, Ascomycota.

The main species of cucurbits cultivated in Mexico are squash (*Cucurbita* spp.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and 'chayote' (*Sechium edule* (Jacq.) Sw.) (SIAP 2020). An important percentage of cucurbit crops are for local consumption. At the same time, the export of these cucurbits in 2018 represented profits of more than 699 million dollars for Mexico (SADER-SIAP 2019). A common and severe disease in cucurbits (Lebeda et al. 2018) is powdery mildew caused by different species of Erysiphales (Ascomycota). It is characterized by the presence of mycelial growth and conidia with a white and powdery appearance, present in patches or completely covering leaves and stems, sometimes appearing on flowers and fruits (Braun & Cook 2012). Erysiphales infection stunts growth and promotes premature desiccation of the leaves, and consequently, decreases the quality and yield of the fruits (Lebeda et al. 2016). Two main species of fungi, *Golovinomyces orontii* (Castagne) V.P. Heluta and *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, have been identified among the causes of powdery mildew (Křístková et al. 2009, Braun et al. 2019). Both Erysiphales species are present in all cucurbits producing areas of the world (Pirondi et al. 2015, 2016).

The pathogenicity and virulence of *Podosphaera xanthii* isolates are highly variable (Bardin et al. 1997, Rabelo et al. 2017, Lebeda et al. 2018). Several pathotypes have been defined according to their infective capacity on differential hosts, which include the species and genera of cucurbits with greater agricultural importance (Bardin et al. 1997, Lebeda et al. 2008). Various physiological races have been determined based on the levels of virulence or severity. Severity levels are evaluated by inoculating *Cucumis melo* (melon) plants, whose varieties have genotypes of differential resistance to the pathogen (Lebeda et al. 2016, 2018, Rabelo et al. 2017, Hong et al. 2018). The severity of the disease is estimated based on the percentage of the foliar area affected by the powdery mildew. Although the severity can be calculated by visual examination (Lebeda et al. 2004), the results present errors and inconsistencies since they depend a lot on the experience and subjectivity of the evaluator(s) (Barbedo 2016, Bock et al. 2020).

Several quantitative techniques have been adapted to assess the severity of plant diseases (Singh et al. 2020). A group of methods uses the properties of pixels in digital image analysis by automatically segmenting islands of pixels, in the visible spectra (Barbedo 2013, Hitimana & Oubong 2014) and in the invisible (Schneider et al. 2012, Al-Qarallah et al. 2017). This strategy has been used to quantify the severity of *P. xanthii* (as *Sphaerotheca fuliginea*) infection in cucumber leaves (*C. sativus*) (Kampmann & Hansen 1994). Another group of methods is based on the measurement of infected areas. In this case, the areas to be measured are delimited manually and then the number of pixels in that area is quantified. For example, to measure the susceptibility of soybeans to infection by *Xanthomonas campestris* pv. *glycines* (Nakano) natural colours were analysed in digital images with the ImageJ program. The "color threshold" function was used to delimit islands of pixels of the same colour and then, areas of various colours were calculated with the "measure" function (Matsunaga et al. 2017). In another example, colour recognition was performed automatically to calculate the leaf area of barley (*Hordeum vulgare* L.) infected by *Blumeria graminis* (DC.) Speer. Photographs were obtained with polarized light and an algorithm was used for the analysis of the images by means of the Quantimet program (Newton 1989). When it is not so easy to delineate the coloured pixels, then the infected areas are digitally coloured. With this approach, the severity of infection of strains of *Podosphaera clandestina* (Wallr.) Lév. was quantified on cherry leaves [*Prunus avium* (L.) L.] with the SigmaScan Pro program (Olmstead et al. 2001). In digital images, the manually coloured area was measured with the "trace area" and "measure area" functions (Olmstead et al. 2001). In another case, the areas were first physically drawn and then, digital images were obtained to quantify the infection by *Colletotrichum destructivum* O'Gara in tobacco leaves (*Nicotiana benthamiana* Domin). Percentages of the diseased leaf area were estimated with the Scion Image program (Wijekoon et al. 2008).

As a first assay, the goal of this study was to explore the applicability of a quantitative technique based on area measurements to estimate differences in the severity of disease caused by *P. xanthii* on leaves of several cucurbits. In this exploratory study, we used digital images of the leaves to directly delimit several polygons and then calculate the sum of the infected areas with the ImageJ program (Schneider et al. 2012). The strategy of demarcation of polygons has not been used with this pathogen previously, so we tested this quantitative technique, to explore whether there are differences in the pathogenicity and severity of infection of two strains of *P. xanthii*.

Material and methods

Plant material

Ten seeds of each of the seven host varieties and species of cucurbits were sown: *C. pepo* (var. 'zucchini' and var. 'criolla'), *Cucurbita ficifolia* Bouché, *Cucurbita okeechobeensis* subsp. *martinezii* (L.H. Bailey) Walters & Deck.-Walt., *C. sativus, C. lanatus* and *S. edule* (Tab. 1). Each plant grew in a plastic pot (2070 cm^3 capacity) with approximately 1700–1800 cm^3 of oak forest leaf soil [Sandy Loam (17.24 % clay, 25.44 % silt, 57.32 % sand), pH 6.41]. The plants (n = 70) developed at room temperature between 14–28 °C (CONAGUA 2021) and natural light. The pathogenicity tests were carried out by inoculating healthy plants between 35 and 40 days old.

Inoculum

Two samples of *Podosphaera xanthii* (M1 and M2) were used for the inoculum. The M1 inoculum came from a crop of 'calabacita criolla' (*C. pepo*) grown in a backyard in central-western Mexico (Chilchota, Michoacán). M2 was obtained from the leaves of a wild 'calabacita' (*Cucurbita radicans* Naudin) growing as a weed in the same geographical area (Purépero, Michoacán). To perform the pathogenicity test, 10 infected leaves were collected from each host plant. The two sets of infected leaves were transported to the laboratory in polyethylene bags and kept at room temperature between 23–25 ºC. The somatic and reproductive structures of the fungi were observed in an optical microscope, to corroborate the identity of *P. xanthii*, by the presence of fibrosin bodies and conidial chains (Braun & Cook 2012). Fungal vouchers for M1 (on leaves of *Cucurbita pepo*; Loc. Ichán, Mpio. Chilchota, Michoacán, México. 11 Sep. 2020, *R. Gregorio-Cipriano 694*) and M2 (on leaves of *Cucurbita radicans*; Loc. El Plan, Mpio. Purépero, Michoacán. Mexico, 10 Sep. 2020, *R. Gregorio-Cipriano 693*) were deposited in the mycological collection in XAL.

Pathogenicity tests

Each of the two inocula of *P. xanthii* (M1 and M2) were used to infect two to three plants (two to four leaves per plant) of the seven host species or varieties of cucurbits. Each inoculum was seeded in a subtotal of 56 leaves. In total 112 leaves were in-

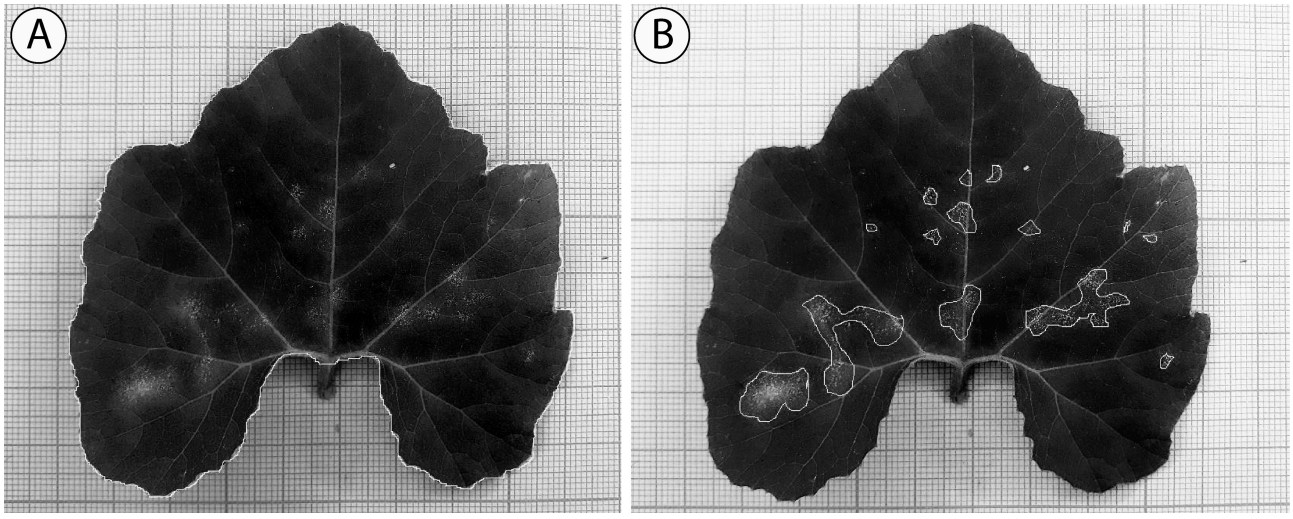

Fig. 1. A) *Cucurbita ficifolia* leaf with powdery mildew. The white line indicates the delimitation of the perimeter of the leaf. B) Delimitation of the infection area of *Podosphaera xanthii*.

oculated. Inoculation was carried out by transferring conidia along the mid vein of the adaxial side of healthy leaves from diseased leaves with a brush (Fonseca et al. 2019). The age of the leaves ranged from the uppermost young leaves, to the medium-aged leaves from the middle of the shoot. Subsequently, the plants were covered with a polyethylene bag for 24 h to maintain high relative humidity and ambient temperature between 23–25 ºC, to allow the establishment of the pathogen. As a negative control, two or three plants of each species and variety were placed without inoculum under the same conditions. After 24 h, the inoculated and non-inoculated plants were placed in a 56 × 73 × 195 cm portable greenhouse without a fan (Home Complete 514537FXW), at a temperature of 14–39 °C and a relative humidity of 45–99 % (Hydrometer and AcuRite 00613 Digital Thermometer). These conditions prevented the exposure of the plants to other sources of Erysiphales inoculum or other pathogens or pests, or the dispersal of the conidia within the greenhouse. Natural ventilation was allowed to avoid excess moisture, while being protected from gusts of wind or rain. The pathogenicity of M1 and M2 in the seven host species or varieties of cucurbits was determined by observing the presence or absence of powdery mildew colonies 15 days after inoculation.

In a separate experiment, we inoculated 12 leaves to estimate the range of conidia transferred by the brushing method. Variation in the inoculum brushed from a diseased leaf to the healthy leaf was quantified by counting the conidia in a total of 36 samples. Samples were obtained by applying a transparent tape onto the diseased (brushed) host leaf and removing it with the conidia attached to the sticky surface of the tape. For each leaf, we counted the numbers of conidia in three separate squares (1x1 cm), under a light microscope.

Measurement of areas

Eighty digital photographs of leaves of infected hosts were obtained. A millimetre sheet was placed as a background for reference to the scale. The ImageJ program (Schneider et al. 2012) was used to quantify total leaf areas, the sum of infected areas, and the area of a scale. Areas were quantified by counting the pixels within the delimited perimeters of the leaf and of the colonies of powdery mildew infection (Fig. 1, and Supplementary Fig. 1). The images were prepared to improve the visibility of the edges of the white spots of the powdery mildew by using the function "Image> Adjust> Brightness / contrast> Auto". In the main menu "Analyze> Set Measurements> Area, Display Label" was selected to delineate three areas separately. First, in each image, a box was delimited with the "Rectangle" tool to measure the area for the scale. Second, the separate polygons of all the powdery mildew spots on a leaf were delimited with the "Free hand selections" tool to measure the infected area. Third, the outline of the leaf was delimited with the "Free hand selections" tool to measure the total leaf area. Measurements of the scale area, the powdery mildew and the whole leaf were made with the function in the

main menu "Analyze> Measure". The same procedure of three separate measurements was applied for each image. The three areas were recorded in pixels and the conversion to millimetres was subsequently carried out using the number of pixels in the 1 cm square as a scale reference in each image. The values of the area of the spots and the total area of the leaves were used to calculate the percentage of infection (PI) of each leaf.

Statistical analysis

Variation of the inoculum brushed from a diseased leaf to the healthy leaf was inspected with the univariate summary statistics and the Shapiro-Wilk test for normality from the numbers of conidia in 36 samples, using PAST statistical package (Hammer et al. 2001). This analysis would provide insight if the inoculum transferred to infect the leaves was consistent among the seven hosts in the comparative experiment.

The host plants, jubilee watermelon (*Citrullus lanatus*) and smooth green 'chayote' (*Sechium edule*) did not show symptoms or signs of powdery mildew. Therefore, they were not considered in the statistical comparisons of the severity values. The variation of the infection percentage values (PI) of each leaf was examined to reveal differences in the severity of the M1 and M2 inocula using analysis of variance in the PAST statistical package (Hammer et al. 2001). A first analysis compared the severity levels of the *P. xanthii* inoculum from the cultivated host (M1, *C. pepo*) among the five infected hosts. The comparison of the means and variances of the PI values was carried out with an analysis of variance (ANOVA), with the option "several sample test". Tukey's test was used for the paired comparison of the five PI averages from M1. In the same way, the five severity levels (PI) of the inoculum of *P. xanthii* from the wild host (M2, *C. radicans*) were compared with a second analysis of variance using the same option "several sample test". These two analyses made it possible to evaluate whether the severity caused by M1 and M2 is the same or not, depending on the five hosts.

To reveal if there are differences in the severity of the M1 or M2 inoculates of *P. xanthii,* we also compared the two values of PI in the same species or variety of infected cucurbit. An analysis of variance (ANOVA) of the two PI values was carried out, separately for each inoculated species of cucurbits. The five paired comparisons of the PI values, by M1 and by M2, were estimated with an analysis of variance using the "two-sample test" option in PAST.

Results

Pathogenicity tests

Univariate summary statistics from the numbers of conidia in 36 samples are presented in supplementary Tab. S1. The quantification of the inoculum brushed from an infected leaf to the healthy leaf reveals a wide range of variation in the number of conidia in a square centimeter (mean=5403, standard error=502, standard deviation=3012). In the 36 samples, most of the conidial counts are to the left of the mean (kurtosis=0.49). The Shapiro-Wilk test (W=0.93, p(N)=0.03) suggests the sample has a distribution significantly different from Normal (Supplementary Fig. 2).

Both inocula of *P. xanthii*, both M1 from the cultivated host (*C. pepo*) and M2 from the wild host (*C. radicans*), were pathogenic on the same five species or varieties of the seven hosts that were infected (Tab. 1, Fig. 2).

Tab. 1. Cucurbit hosts for pathogenicity tests with *Podosphaera xanthii* and pathogenicity of two inocula of *Podosphaera xanthii*, from a cultivated species (MI) and a wild one (M2).

Species	Common name or variety	Kind	M1 (*C. pepo*)	M2 (*C. radicans*)
1 *Citrullus lanatus*	Jubilee watermelon	Commercial[1]	–	–
2 *Cucumis sativus*	Poinsett cucumber	Commercial[2]	+	+
3 *Cucurbita ficifolia*	'Chilacayote'	'Criolla'[3]	+	+
4 *Cucurbita pepo*	Zucchini squash	Commercial[2]	+	+
5 *Cucurbita pepo*	'Calabacita criolla'	'Criolla'[3]	+	+
6 *Cucurbita okeechobeensis* subsp. *martinezii*	Butternut squash	Wild[4]	+	+
7 *Sechium edule*	Smooth green 'chayote'	Commercial[5]	–	–

[1] Supplier: Rancho Los Molinos; [2] Supplier: Germinal; [3] Chilchota, Mich.; [4] Xalapa, Ver.; [5] Mercado de abastos, Zamora, Mich. (+) = pathogen; (–) = non-pathogenic

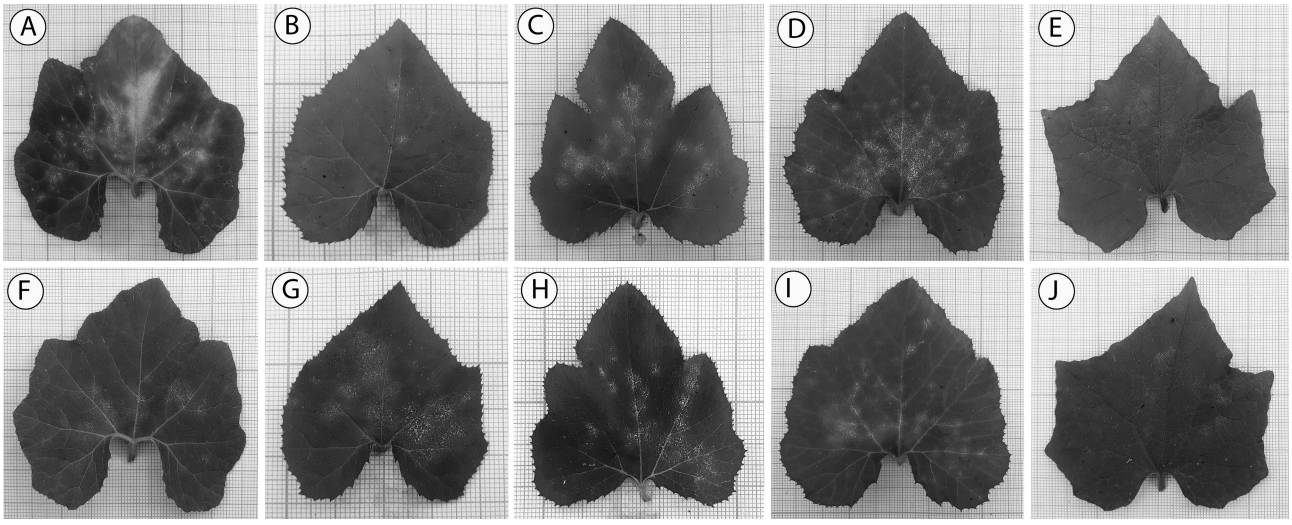

Fig. 2. (A–J) Leaves of cucurbits susceptible to *Podosphaera xanthii*. Each photograph is representative of the average values of severity levels (PI). Above: species infected with inoculum of the cultured host *Cucurbita pepo* (M1). Bottom: species infected with the inoculum of the wild host *C. radicans* (M2). (A, F) *Cucurbita ficifolia*; (B, G) *C. okeechobeensis subsp. martinezii*; (C, H) *C. pepo* var. 'criolla' (D, I); *C. pepo* var. 'zucchini' and (E, J) *Cucumis sativus*.

Severity levels

The first analysis of variance (ANOVA) revealed that the five means of severity (PI) caused by M1 are not different (P = 0.0929), among the five susceptible cucurbits (Tab. 2). In contrast, the second analysis of variance suggests that there are differences between the five means of infection severity (PI) caused by *P. xanthii* from the wild host M2 (P = 0.0045). The mean value of the infection was significantly lower in *C. ficifolia* with respect to *C. okeechobeensis* subsp. *martinezii* and *C. pepo* (Tab. 3).

The five analyses of variance for the paired comparisons of the means of the severity values (PI) caused by M1 with respect to M2 showed that there are significant differences in only two of the five susceptible cucurbits (Fig. 3). The severity (PI) caused in *C. ficifolia* by M1 was significantly higher than the PI caused by M2 (P = 0.0065). The severity (PI) by M2 in *C. okeechobeensis* subsp. *martinezii* was higher than by M1 (P = 0.0002).

Discussion

This study with digital images has revealed the advantage of using a widely available technique to quantify the pathogenicity and virulence of *Podosphaera xanthii*. Although a destructive method was used, since the digital images were taken from detached leaves, it could also be implemented on leaves *in situ* to monitor the progress of the infection. The area quantification used does not depend on the different tones in the digital image by pixel islands and measuring in the visible spectrum (Barbedo 2013, Hitimana & Oubong 2014) and invisible spectrum (Schneider et al. 2012, Al-Qarallah

Tab. 2. Analysis of variance and Tukey's test for the paired comparison of means of severity (PI) of the inoculum of *P. xanthii* from *C. pepo* (M1) in the five susceptible species.

Hosts	*C. ficifolia*	*C. okeechobeensis* subsp. *martinezii*	*C. pepo* ('criolla')	*C. pepo* ('zucchini')	*C. sativus*
Cucurbita ficifolia		0.3337	0.9929	0.9999	0.922
C. okeechobeensis subsp. *martinezii*	2.694		0.16	0.2736	0.0699
C. pepo ('criolla')	0.5996	3.294		0.9982	0.9945
C. pepo ('zucchini')	0.176	2.87	0.4237		0.9557
C. sativus	1.162	3.856	0.5628	0.9865	

Above: values of statistical significance (P = probability of equality). Below: Q values (critical value from Tukey's table).

Tab. 3. Analysis of variance and Tukey's test for the paired comparison of means of severity (PI) of the inoculum of *P. xanthii* from *C. radicans* (M2) in the five susceptible species.

Hosts	*C. ficifolia*	*C. okeechobeensis* subsp. *martinezii*	*C. pepo* ('criolla')	*C. pepo* ('zucchini')	*C. sativus*
Cucurbita ficifolia		*0.0133	*0.0034	0.1268	0.1871
C. okeechobeensis subsp. *martinezii*	4.828		0.9866	0.8681	0.7692
C. pepo ('criolla')	5.538	0.7104		0.5887	0.4647
C. pepo ('zucchini')	3.46	1.367	2.078		0.9996
C. sativus	3.176	1.652	2.362	0.2845	

Above: values of statistical significance (*P* = probability of equality). Below: Q values (critical value from Tukey's table). Different averages are indicated with an asterisk (*).

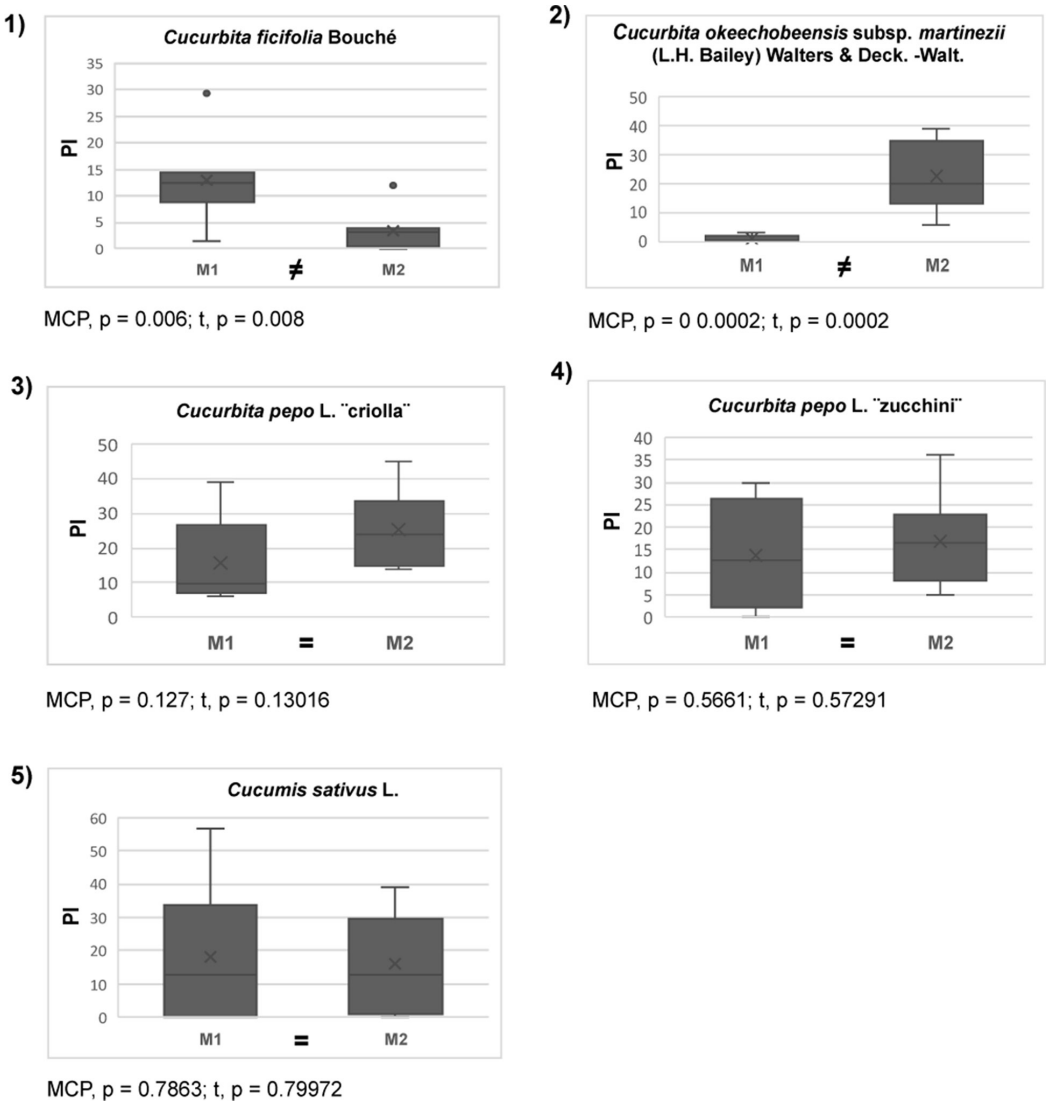

Fig. 3. Paired comparison of the severity (PI) caused by the inocula of *Podosphaera xanthii*, M1 and M2 in the five susceptible cucurbits. Below each graph, the statistical significance values (*P*) obtained with the Monte Carlo Permutation test (MCP) and with the T-test (t) in the analysis of variance (ANOVA) are shown. The "equal" or "different" sign is included in the graph depending on the result of each comparison through each analysis of variance.

et al. 2017). Therefore, no effects are introduced by variations in lighting or non-standardized colour capture. The technique used here relies on manually tracing infection polygons, leaf perimeters, and scale, making it easy to measure areas. Certainly, a study remains to be designed to determine the level of error in this operation. What has become evident in this preliminary experiment is the speed in the registration of the areas, so that in future studies the number of samples may be expanded. The availability of the image collection also creates the opportunity to repeat the quantitative estimates of the infected areas. Therefore, the use of this method of quantification of infected areas with the use of digital images is recommended for later larger studies of the pathogenicity of *P. xanthii*.

This initial study made it possible to detect the pathogenicity and virulence of *P. xanthii* in seven species and varieties of Cucurbitaceae. The two inocula of this fungus, one from the cultivated species (M1) and the other from a wild one (M2), were pathogenic in the same five cucurbits. In *P. xanthii*, several pathotypes have been identified according to the variation in its infective capacity on different hosts. Our results suggest that the two evaluated inocula could belong to the same pathotype since they infect the same five species. The differences observed in the severity of virulence measured with the infected area suggests that the two inocula could correspond to different races. However, to corroborate these inferences, more pathogenicity tests need to be carried out including different cultivars of *Cucumis melo*. This species has a great variety of genotypes, some of which are included in the standard set of differentials to determine pathotypes and races of powdery mildew in cucurbits (Lebeda et al. 2008, Hong et al. 2018).

Another informative result is that the two inocula of *P. xanthii* (M1 and M2) did not infect two cultivated species, *C. lanatus* (watermelon) and *S. edule* ('chayote'). This result does not appear to be related to the possible lack of consistency in the amount of conidia transferred by the brushing method. Our quantification of the conidia input to infect leaves suggest that the brushing method transfers a minimum of 1000 conidia/cm^2 (Supplementary Figure 2), which was enough conidia to cause an infection in the other hosts. Rather, the explanation may be found in the interaction of possible races and the two uninfected hosts. In some Asian countries and in the USA, there are records of powdery mildew in both crops caused by *P. xanthii* (Farr & Rossman 2021). Recently, two races (1W and 2W) of a *P. xanthii* pathotype were discovered in the United States, capable of infecting *C. lanatus* very aggressively (Davis et al. 2001, 2007; Tetteh et al. 2010). These two races have dispersed throughout different regions of that country (Kousik et al. 2019). In Mexico, there are no records of significant outbreaks of powdery mildew in *C. lanatus* crops. Therefore, the fact that this cucurbit was not susceptible to M1 and M2 could indicate that these inocula are different from the two American races (1W and 2W). The absence of infection in *S. edule* could also suggest that the two inocula used in the present study (M1, M2) are also different from the powdery mildew caused by *P. xanthii* on *S. edule* documented in other parts of the world (Farr & Rossman 2021) and another inoculum of *P. xanthii* registered in Mexico. In a recent exploration, a small *P. xanthii* infection was found in a *S. edule* plant in a commercial crop from the state of Veracruz, but not in other parts of Mexico (Gregorio-Cipriano et al. 2020). Powdery mildew in *S. edule* is common in Mexico and is considered one of the most frequent diseases of the crop (Olguín-Hernández et al. 2011). But in this country the causal agent is *Neoerysiphe sechii* R. Gregorio-Cipriano and D. González (Gregorio-Cipriano et al. 2020). The absence of infection in *C. lanatus* and *S. edule* plants inoculated with *P. xanthii* (M1 and M2), present in central-western Mexico (Chilchota, Michoacán), could indicate that these inocula are different from powdery mildew caused by *P. xanthii* in other parts of the world.

The severity (PI) caused by M1 was not different between the five inoculated hosts in this study. The level of severity does not depend, apparently, on the differences between these species and varieties of cucurbits. There was no discrepancy between the four cultivated species and the inoculated wild species. In contrast, the severity of *P. xanthii* from the wild host (M2) varied among some of the cucurbits. The paired comparisons of the PI values of M1 and M2 in each species or variety of cucurbits allowed us to detect that the severity is different in two of the five susceptible cucurbits. *Podosphaera xanthii* from the cultivated host (M1) caused greater damage in *C. ficifolia*, a cultivated species, than the inoculum from the wild host (M2). On the other hand, the inoculum from the cultivated host (M1) caused less damage in the wild species (*C. okeechobeensis* subsp. *martinezii*) than the inoculum of *P. xanthii* from the wild host (M2). This suggests a distinctive virulence between both inocula of *P. xanthii*. However, to determine whether they are two physiological races, it is necessary to use monosporic cultures of the fungus and measure its severity in the differ-

ential plants of *Cucumis melo* as conventionally used for this purpose (Lebeda 2016, 2018).

Paired comparisons of the means of the PI values of M1 and M2 were only significant in *C. sativus* and *C. okeechobeensis* subsp. *martinezii*. The results showed that the maximum percentage of infection area (PI) obtained for M1 was 58 % in *C. sativus* and 45 % for M2 in *C. okeechobeensis* subsp. *martinezii*. In all cases, the observed severity levels of both *P. xanthii* inocula are interpreted as low to moderate in the five susceptible cucurbit species. The severity values observed are at a level equal to or less than level three (40–69 % of PI) of the five established by Davis et al. (2001) visually, in six replicas of the total plant of *C. lanatus* (leaf, stem and cotyledon) of approximately 100 accessions. This quantification with digital images allowed acquiring results comparable to those obtained by Davis et al. (2001) with visual methods. Clearly, it is better to use a quantitative technique to estimate the pathogenicity and virulence of *Podosphaera xanthii*.

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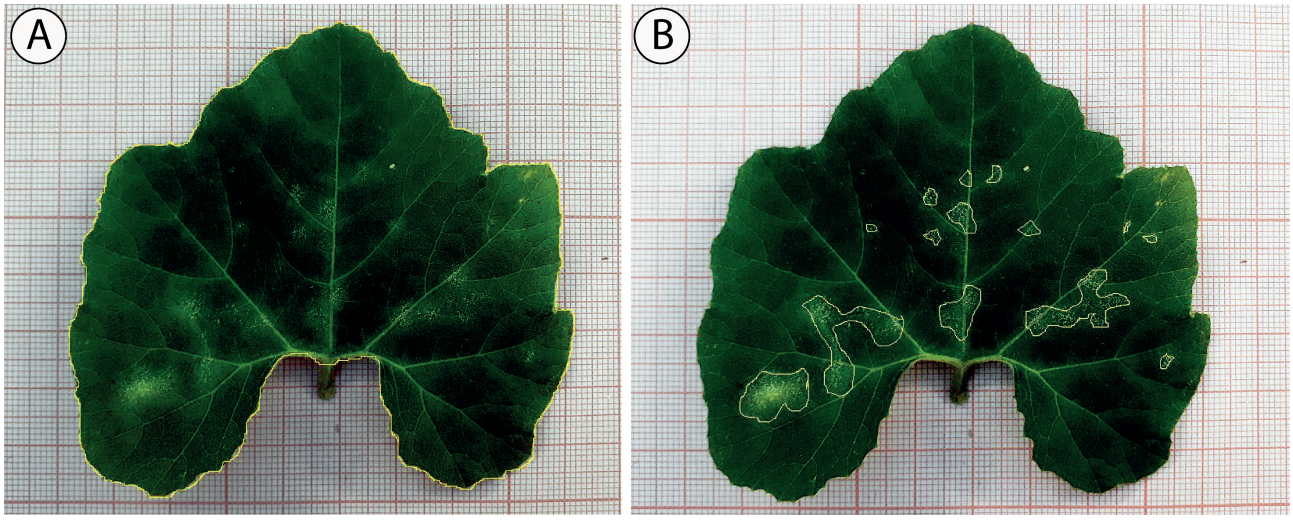
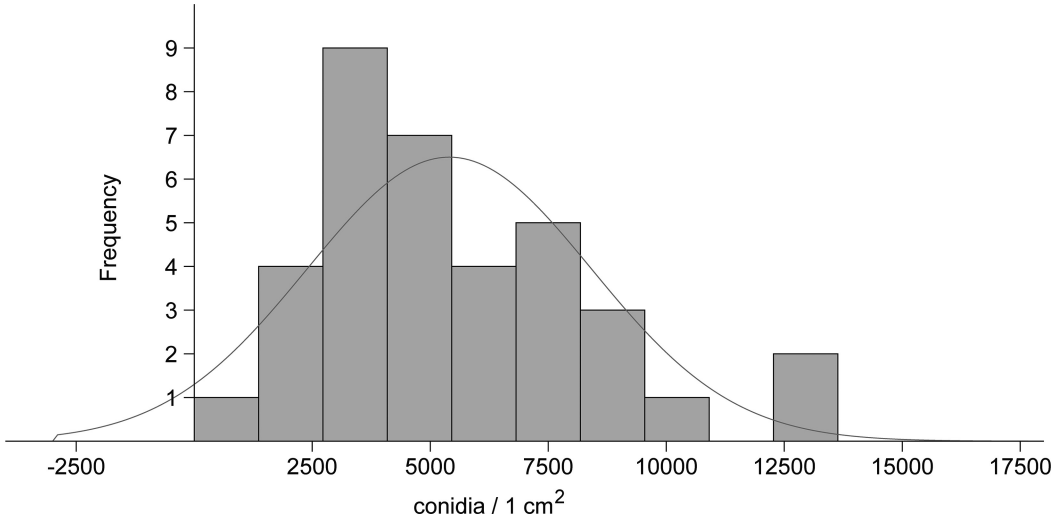
Tab. S1. Variation of the inoculum brushed from an infected leaf to the healthy leaf estimated by 36 counts of conidia in a square centimeter.

N	36
Min	1134
Max	13300
Mean	5403.806
Std. error	502.0113
Stand. dev	3012.068
Median	4729.5
Skewness	0.888169
Kurtosis	0.492538
Coeff. var	55.73975



Supplementary Fig. 1. Color version of Fig. 1



Supplementary Fig. 2. Histogram and normal fit of the number of conidia in a square centimeter to quantify the inoculum brushed from an infected leaf to the healthy leaf. The frequency distribution of 36 counts is significantly different from Normal (Shapiro-Wilk test W=0.93, p(N)= 0.03).