

## ASSESSMENT OF DIMENSIONS IN ORDER TO AID CLASSIFICATION OF EUROPEAN RED MITE AND TWO-SPOTTED SPIDER MITE BY MEANS OF DIGITAL IMAGING OF INFECTED LEAVES

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### ABSTRACT

The methods currently used to assess orchard infestation are time-consuming and do not take into account non-adult forms of mites, due to their small size. Advance in digital imaging technology, however, has resulted in the discovery of a more viable method to enable a fast and reliable assessment of orchard infestation. Digital images of infected apple leaves were made and examined for the presence of European red mites and two-spotted spider mites. As well as adult mites, non-adult European red mite forms were also encountered. After extracting all objects considered as mites from the image, imaging software was then used to assess their dimension and shape parameters. Length, width, area, and equivalent diameter were different for all three observed mite groups: adult female European red mites, possible non-adult European red mites and adult two-spotted spider mites. Differences in circularity and elongation were found in adult two-spotted spider mites and various forms of European red mites, while the perimeter was similar in adult European red mites and two-spotted spider mites, and different in the non-adult forms of European red mites. However, as the ranges of 95% prediction intervals for these features overlap, a combination of at least two features as well as employing statistical procedures may greatly increase the probability of appropriate classification of the observed objects as different mites.

## Introduction

Spider mites are secondary pests in apple orchards, and with a lack of natural enemies, are in a position to build numerous populations, causing significant losses in the yield and quality of apples. The basis for making protective treatments against these pests is to compare the severity of their occurrence with an economic injury level. According to current recommendations for Polish orchards, spider mites should be controlled when their population exceeds 3 motile forms per leaf, in the period up to mid-July, and 5-7 motile individuals in the latter part of the season. The difficulty in assessing the number of mites with any

degree of accuracy is that they are tiny organisms. The length of the adult female two-spotted spider mite varies between 0.42 and 0.58 mm, with a mean value of 0.53 mm (Boczek, 1999). Similarly, Meena et al., (2013) found that adult females measured 0.421-0.686 mm (with a mean of  $0.472 \pm 0.016$  mm) in length and 0.226-0.417 mm (with a mean of  $0.271 \pm 0.08$  mm) in width. However, according to Clotuche et al., (2012) the mean body length of adult females is only  $0.348 \pm 0.047$  mm. In the case of European red mite (*Panonychus ulmi* Koch), the length of the adult female body varies between 0.32 and 0.37 mm (Newcomer and Yothers, 1929), but some researchers report, however, that it is about 0.4 mm (Considine, 1995). The length of the male is even shorter, between 0.27 and 0.3 mm (Newcomer and Yothers, 1929), but due to their less frequent occurrence, the distinction between male and female forms is of lesser importance. The length of the larva of two-spotted spider mite is between 0.15-0.20 mm, protonymph approx. 0.23 mm and deutonymph 0.25-0.30 mm (Boczek, 1999).

In field conditions, the precise determination of this pest population is not feasible with the naked eye, and requires the use of a microscope. There are various sampling methods to assess the number of mites (Sabelis, 1985), one of which is counting the mites under a stereoscopic microscope on previously picked leaves. Employing this method means that monitoring an orchard with an area of 5 hectares would result in the assessment of 200 leaves; without doubt, a time-consuming and costly process. Among other recognized techniques are leaf-washing methods (Henderson, 1960; Zacharda et al., 1988) or sampling methods, using a mite-brushing machine (Henderson and McBurnie, 1943). Although these methods can save time, they are not absolutely reliable, as not all individuals are removed from the leaf surface and, in addition, in the case of the mite brushing method, it is necessary to use a special machine. Therefore, due to the problems of adequate assessment of the finite population of mites on the plant leaves, some attempts have been made to use photos of infested leaves (Asquith, 1965; Sircom, 2000).

The microscopic methods currently used to assess the orchard infestation are sufficiently precise when considering adult forms of mites, while the assessment of non-adult mite forms, due to their smaller sizes, is much more difficult, and far less reliable. The colouring patterns of plant leaves make it even more difficult, especially when reddish spots of a different size and shape are present. In such a case, the human observer routinely uses information relating to the size and shape of the object sought, and for digital image analysis such information must also be provided.

Digital image analysis is already widely used in agriculture, with some analyses depending solely on information relating to colour, whilst others focus on determining size and shape. For example, assessing size and morphological features is used in the commercial classification of cereal grains (Majumdar and Jayas, 2000; Emadzadeh et al., 2010), in research on plant tissue cell structure (Konstankiewicz et al., 2001) and in the identification of soil macropores to aid the prediction of water flow through soil (Czachor and Lipiec, 2004).

Although other means of assessing insect population are available, some employing the analysis of images of insects on sticky trap paper, such as Scoutbox by SoilCares, the Netherlands and iMetos iScout by Pessl, Austria, the reliability of assessment and details of image analysis algorithms of such methods are not known. Moreover, such devices are specifically dedicated to monitor much larger flying insects.

The aim of the presented study is to analyse the feasibility of using digital imagery to aid the identification of selected mite groups. The research focused on female adult forms of European red mite (*Panonychus ulmi*) and two-spotted spider mite (*Tetranychus urticae*), as well as objects, which are difficult to classify, but could well be non-adult mite forms of European red mite.

## Materials and methods

Digital images of leaves were taken in 2012 and 2013 with a Canon EOS 500D camera supplied with the lens microcorrected to the image sensor. The camera matrix size was 17.9 Mpix. The camera was mounted on a vertical stand with adjustable height of a camera grip. The height of the camera was adjusted to cover approximately 1/4 to 1/3 of the leaf area. In order to cover the whole leaf area, four images of each leaf were made. The leaves were covered with glass to flatten any natural wrinkles in the leaf blade and to reduce the required depth of focus. Millimetre graph paper was placed below the leaf, to assess the real pixel density and calibrate the images of mites. The illumination of 3400 K colour temperature was used.

Fresh apple leaves were used for artificial infestation of female European red mites, in a quantity of between 3 and 20. Each time new leaves were collected from the orchard. Some leaves were naturally infested with two-spotted mites. As well as adult female mites, some non-adult forms of mites were also applied when available. Digital images of the apple leaves were made from 19 July until 18 August during the 2012 season and from 12 July until 6 September during the 2013 season. Altogether, images of 39 leaves in 2012 and 31 leaves in 2013 were made. The mites present on the leaf images were classified into three groups: A) adult female European red mite (*Panonychus ulmi*), B) possible European red mite (e.g. a protonymph or deutonymph stage), and C) adult two-spotted spider mite (*Tetranychus urticae*). As the classical binocular methods for assessing orchard infestation do not distinguish different non-adult mite forms, the analyses of digital images followed the same pattern. The objects present on images and marked as mites were extracted from leaf images using a selection of similar pixels feature of Adobe Photoshop Elements versions 8 and 11.

NIS Elements software, version BR 3.22, was used to assess the dimension and shape parameters of each object. The following features were measured (Nikon Imaging System):

Length – expressed as maximal Feret diameter (MaxFeret), i.e. maximal value of projected length of object at angle 0, 10, 20, 30, ..., 180 (Herdan and Smith, 1953).

Width – expressed as minimal Feret diameter (MinFeret), i.e. minimal value of projected length of object at angle 0, 10, 20, 30, ..., 180 (Herdan and Smith, 1953). Area – the sum of pixels calibrated in area units

Equivalent diameter (EqDiameter) – a size feature derived from the area, which determines the diameter of a circle with the same area as the measured object

$$EqDiameter = \sqrt{\frac{4 * Area}{\pi}} \quad (1)$$

Perimeter – the measure of total boundary, which is calculated from four projections in the directions 0, 45, 90 and 135 degrees using Crofton's formula:

$$Perimeter = \frac{\pi * (Pr_0 + Pr_{45} + Pr_{90} + Pr_{135})}{4} \quad (2)$$

Circularity – a measurement of shape, which equals to 1 only for circles, and is calculated according to the formula:

$$Circularity = \frac{4 * \pi * Area}{Perimeter^2} \quad (3)$$

Elongation - calculated according to the formula:

$$Elongation = \frac{MaxFeret}{MinFeret} \quad (4)$$

Prior to statistical analyses, the outliers of either length or width had been excluded. Outliers were selected according to Tukey (1977), as values holding one of the following conditions:

$$\text{data point value} > UQ + 1.5 * (UQ - LQ) \quad (5)$$

$$\text{data point value} < LQ - 1.5 * (UQ - LQ) \quad (6)$$

where:

UQ = upper quartile

LQ = lower quartile

Statistical analyses were conducted by means of Dell Statistica (data analysis software system), version 13.

## Results and discussion

In the 2012 season, 150 adult female European red mites were identified, with an additional 69 possible European red mites, and 24 adult two-spotted spider mites. After the removal of outliers 143, 68 and 22 mites were left, respectively. In the 2013 season a total of 226 adult female European red mites were identified, with a further 89 possible European red mites, and no adult two-spotted spider mites. After the removal of outliers 225 and 89 mites were left, respectively. Due to lack of artificial infestation (only natural) of leaves with two-spotted mites, the number of observed *T. urticae* mites was low, and contrary to *P. ulmi*, no non-adult *T. urticae* were observed. The obtained picture densities varied between 69.85 and 98.45 pixels per millimetre.

The basic dimension features, such as length, width and area (Table 1) were significantly different for all distinct mite groups. The observed ranges for the length of adult mites (Table 3) were within the ranges given by Newcomer and Yothers (1929) and Considine (1995) in the case of the female European red mite, and by Clotuche et al., (2012) and Meena et al., (2013) in the case of the two-spotted spider mite. The adult female European red mites were characterized by a larger area and width, while the adult two-spotted spider mites displayed the longest mean length. Considering all the above features, the objects classified as possible European red mites were the smallest ones. The equivalent diameter (Table 1), which is calculated using the area, is also significantly different for all mite groups, the largest being for the adult female European red mites and the smallest for possible European red mites. The circularity and elongation estimated for objects classified as adult female European red mites and possible European red mites did not distinguish be-

tween these two classes of object, however, they differ significantly when compared to adult two-spotted spider mites (Table 1).

Table 1.  
*Basic statistical characteristic of the considered size and shape features for the investigated mite groups*

Feature	Mite group	Mean <sup>[a]</sup>	Median	Standard deviation	Standard error	Valid N
Length (mm)	A) adult female European red mite	0.386 b	0.386	0.0314	0.00164	368
	B) possible European red mite	0.295 a	0.297	0.0476	0.00380	157
	C) adult two-spotted spider mite	0.408 c	0.406	0.0554	0.01181	22
Width (mm)	A) adult female European red mite	0.253 c	0.252	0.0202	0.00106	368
	B) possible European red mite	0.194 a	0.195	0.0271	0.00216	157
	C) adult two-spotted spider mite	0.217 b	0.225	0.0238	0.00507	22
Area (mm <sup>2</sup> )	A) adult female European red mite	0.074 c	0.073	0.0104	0.00054	368
	B) possible European red mite	0.044 a	0.044	0.0122	0.00098	157
	C) adult two-spotted spider mite	0.069 b	0.072	0.0151	0.00322	22
Equivalent diameter (mm)	A) adult female European red mite	0.306 c	0.305	0.0218	0.00114	368
	B) possible European red mite	0.234 a	0.235	0.0337	0.00269	157
	C) adult two-spotted spider mite	0.295 b	0.303	0.0330	0.00703	22
Perimeter (mm)	A) adult female European red mite	1.024 b	1.017	0.0923	0.00481	368
	B) possible European red mite	0.780 a	0.789	0.1181	0.00943	157
	C) adult two-spotted spider mite	1.027 b	1.049	0.1405	0.02996	22
Circularity (-)	A) adult female European red mite	0.885 a	0.899	0.0720	0.00375	368
	B) possible European red mite	0.890 a	0.896	0.0558	0.00445	157
	C) adult two-spotted spider mite	0.823 b	0.824	0.0595	0.01268	22
Elongation (-)	A) adult female European red mite	1.53 a	1.52	0.113	0.0059	368
	B) possible European red mite	1.52 a	1.5	0.177	0.0141	157
	C) adult two-spotted spider mite	1.89 b	1.86	0.230	0.0489	22

<sup>[a]</sup> Means followed by different letters within each feature are significantly different at  $p \leq 0.05$  according to Duncan's test.

There were also significant differences between the widths measured in season 2012 and 2013 for adult European red mites (group A) and objects classified as possible European red mites (group B) (Table 2). The seasonal difference in width also resulted in a significant impact on the calculated values of perimeter, circularity and elongation for each season.

Table 2.  
*The effect of observation by season on the characteristic of selected size and shape features for different mite groups*

Feature	Mite group	Year	Mean <sup>[a]</sup>	Standard deviation	Standard error	Valid N
Width (mm)	A) adult female European red mite	2012	0.248 a	0.0184	0.00154	143
		2013	0.256 b	0.0207	0.00138	225
	B) possible European red mite	2012	0.184 a	0.0279	0.00339	68
		2013	0.202 b	0.0238	0.00253	89
Perimeter (mm)	A) adult female European red mite	2012	1.005 a	0.0717	0.00599	143
		2013	1.037 b	0.1015	0.00677	225
	B) possible European red mite	2012	0.752 a	0.1375	0.01668	68
		2013	0.801 b	0.0963	0.01020	89
Circularity (-)	A) adult female European red mite	2012	0.909 b	0.0359	0.00300	143
		2013	0.870 a	0.0840	0.00560	225
	B) possible European red mite	2012	0.908 b	0.0547	0.00663	68
		2013	0.876 a	0.0529	0.00561	89
Elongation (-)	A) adult female European red mite	2012	1.56 b	0.127	0.0106	143
		2013	1.51 a	0.097	0.0065	225
	B) possible European red mite	2012	1.57 b	0.214	0.0259	68
		2013	1.48 a	0.131	0.0139	89

<sup>[a]</sup> Means followed by different letters within each feature are significantly different at  $p \leq 0.05$  according to Duncan's test.

Table 3.  
*Minimum, maximum, lower quartile, upper quartile values of particular size and shape features for different mite groups*

Feature	Mite group	Minimum	Maximum	Lower quartile	Upper quartile
Length (mm)	A) adult female European red mite	0.298	0.466	0.365	0.409
	B) possible European red mite	0.185	0.409	0.260	0.328
	C) adult two-spotted spider mite	0.325	0.527	0.369	0.449
Width (mm)	A) adult female European red mite	0.202	0.304	0.241	0.267
	B) possible European red mite	0.139	0.251	0.173	0.217
	C) adult two-spotted spider mite	0.160	0.250	0.203	0.228
Area (mm <sup>2</sup> )	A) adult female European red mite	0.046	0.104	0.066	0.081
	B) possible European red mite	0.020	0.070	0.034	0.054
	C) adult two-spotted spider mite	0.042	0.097	0.060	0.080
Equivalent diameter (mm)	A) adult female European red mite	0.241	0.364	0.291	0.322
	B) possible European red mite	0.158	0.298	0.207	0.262
	C) adult two-spotted spider mite	0.230	0.351	0.276	0.319

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Feature	Mite group	Minimum	Maximum	Lower quartile	Upper quartile
Perimeter (mm)	A) adult female European red mite	0.836	1.496	0.967	1.071
	B) possible European red mite	0.509	1.053	0.682	0.868
	C) adult two-spotted spider mite	0.790	1.276	0.924	1.102
Circularity (-)	A) adult female European red mite	0.340	0.978	0.867	0.925
	B) possible European red mite	0.714	1.000	0.860	0.925
	C) adult two-spotted spider mite	0.721	0.935	0.768	0.881
Elongation (-)	A) adult female European red mite	1.14	1.90	1.46	1.60
	B) possible European red mite	1.14	2.20	1.41	1.61
	C) adult two-spotted spider mite	1.44	2.33	1.73	2.02

All the experimental data was used to establish 95% prediction intervals of all size and shape features for each investigated group of mites (Table 4), providing all features are characterized with normal distribution. For a practical assessment of the presence of mites on the leaves through digital image processing, the parameters processed from the image should fit within the ranges of the values presented in Table 4. However, since the ranges presented for different mite groups overlap considerably, the distinction of different mites is not always obvious. Nonetheless, low values of standard deviations, standard errors (Table 1) and differences in quartile ranges (ranges between lower and upper quartiles – Table 3) suggest that such distinction is possible when an appropriate statistical procedure is used.

Table 4.  
*Estimated limits of the 95% prediction intervals for different size and shape features and individual mite groups*

Feature	Mite group	Lower limit of prediction interval	Upper limit of prediction interval
Length (mm)	A) adult female European red mite	0.324	0.448
	B) possible European red mite	0.201	0.389
	C) adult two-spotted spider mite	0.290	0.526
Width (mm)	A) adult female European red mite	0.213	0.293
	B) possible European red mite	0.141	0.248
	C) adult two-spotted spider mite	0.166	0.267
Area (mm <sup>2</sup> )	A) adult female European red mite	0.0532	0.0943
	B) possible European red mite	0.0195	0.0680
	C) adult two-spotted spider mite	0.0373	0.1010
Equivalent diameter (mm)	A) adult female European red mite	0.263	0.349
	B) possible European red mite	0.167	0.300
	C) adult two-spotted spider mite	0.225	0.365
Perimeter	A) adult female European red mite	0.842	1.206

Feature	Mite group	Lower limit of prediction interval	Upper limit of prediction interval
(mm)	B) possible European red mite	0.546	1.014
	C) adult two-spotted spider mite	0.728	1.326
Circularity (-)	A) adult female European red mite	0.743	1.027
	B) possible European red mite	0.779	1.000
	C) adult two-spotted spider mite	0.697	0.949
Elongation (-)	A) adult female European red mite	1.307	1.753
	B) possible European red mite	1.171	1.872
	C) adult two-spotted spider mite	1.402	2.378

The ranges of 95% prediction intervals for length, area, equivalent diameter and perimeter overlap (Table 4) in the case of adult female European red mites and two-spotted spider mites. But the ranges of 95% prediction intervals for these variables differ for both adult mites and possible European red mites. The elongation feature shows, in turn, only a partial overlap of the ranges of prediction intervals for adult two-spotted spider mites and all forms of European red mites. The prediction intervals estimated for individual size features are not sufficient to enable classification of different mites. However, a combination of at least two features, e.g. width and elongation, as well as employing statistical procedures, may be sufficient for significant increase in the probability of classification of the observed objects as different mites. More effective results could be obtained by optimizing a combination of three features: width and elongation, along with one of the remaining size features, i.e. length, area, equivalent diameter or perimeter.

## Conclusions

The analysed size and shape features might be useful for differentiating European red mites from two-spotted spider mites on digital images of fruit tree leaves. The obtained plausible length (95% prediction interval of maximum Feret diameter) of mites varied between 0.324 and 0.448 for adult female European red mites, 0.201 and 0.389 mm for observed non-adult European red mites and between 0.290 and 0.526 mm for adult two-spotted spider mites. These values generally agree with literature. The described method for the measurement of mites may enable automatic identification of mites on the leaves collected from orchards. Further research should collect more data, with special emphasis on mites other than European red mites, in order to develop a procedure combining different size and shape features for effective classification of objects observed on leaves.



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## **OCENA ROZMIARÓW I PARAMETRÓW KSZTAŁTÓW PRZY UŻYCIU KOMPUTEROWEJ ANALIZY OBRAZU JAKO NARZĘDZIE ROZRÓŻNIANIA PRZĘDZIORKA OWOCOWCA I PRZĘDZIORKA CHMIELOWCA**

**Streszczenie.** Aktualnie używane metody oceny zasiedlenia sadów przez przędziorki są bardzo pracochłonne i dla zachowania precyzji wymagają zastosowania powiększeń osiągalnych za pomocą np. mikroskopu stereoskopowego. Rozwój technologii analizy obrazu stwarza możliwość opracowania szybszej i dokładniejszej metody lustracji sadów. Wykonano zdjęcia cyfrowe liści jabłoni i dokonano oceny ich zasiedlenia przez przędziorka owocowca i przędziorka chmielowca. Oprócz osobników dorosłych przędziorka owocowca brano również pod uwagę formy niedorosłe tego gatunku. Wszystkie obiekty uznane za przędziorki wydzielono ze zdjęć i wykonano ocenę ich rozmiarów i kształtów przy użyciu oprogramowania NIS-Elements. Długość, szerokość, powierzchnia i średnica równoważna różniły się istotnie dla wszystkich badanych grup, identyfikowanych jako: dorosłe żeńskie osobniki przędziorka owocowca, prawdopodobne niedorosłe formy przędziorka owocowca, oraz dorosłe osobniki przędziorka chmielowca. Różnice w kolistości i wydłużeniu wystąpiły w przypadkach dorosłych osobników przędziorka chmielowca oraz różnych formach przędziorka owocowca. Długość perymetru była podobna dla osobników dorosłych przędziorka owocowca i przędziorka chmielowca, natomiast była istotnie mniejsza dla niedorosłych form przędziorka owocowca. Jednocześnie dla wszystkich badanych cech 95% przedziały predykcji pokrywają się lub są częściowo wspólne dla identyfikowanych grup przędziorków. Uzyskane wyniki wskazują, że efektywne rozróżnienie obiektów rozpoznawanych na zdjęciach może wymagać łącznego zastosowania przynajmniej dwóch cech oraz opracowania właściwej procedury statystycznej.

**Słowa kluczowe.** klasyfikacja, przędziorki, *Panonychus ulmi*, kształt, rozmiar, *Tetranychus urticae*

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