Eur J Plant Pathol (2011) 129:21–29
DOI 10.1007/s10658-010-9636-7

Improving sooty blotch and flyspeck severity estimation on apple fruit with the aid of standard area diagrams

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Accepted: 25 May 2010 / Published online: 22 October 2010

Abstract Sooty blotch and flyspeck is caused by numerous species of fungi that colonize the surface of apple fruit and thereby lower its market value. Although this disease poses a substantial threat to apple growers' profitability in some regions, reliable and cost-effective methods for epidemiological and disease control studies have not been validated, nor are they widely available. We modified a standard area diagram to aid sooty blotch and flyspeck severity assessments and quantified its impact on accuracy and precision of visual estimates. Samples of 'Fuji' and 'Mutsu' fruit were photographed both from the top and laterally. Severity was assessed from a sub-sample of 160 images using image analysis software. Validation of the diagram was performed by eight raters who independently assessed severity in two series of selected images representing the lateral view and the top view, initially unaided and subsequently with the aid of the scale. Severity estimates ranged from 0.4% to 98% (most fruit had <10% severity). Accuracy and precision of the estimates were significantly improved when using the diagrammatic scale; concordance correlation coefficient values increased from 0.81 to 0.95. A strong tendency to underestimate severity for the mid-range to high levels was minimized when using the aid, which also improved reproducibility of the estimates among raters. In addition to strengthening evidence that a standard area diagram can be used reliably in sooty blotch and flyspeck studies, we expanded its application to disease assessment in the peduncle region, which enhances the usefulness of the method for evaluating efficacy of management practices.

Keywords Loss assessment · *Malus* x *domestica* · Lin´s CCC

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Introduction

Sooty blotch and flyspeck (SBFS) is a disease complex caused by more than 60 species of epiphytic fungi that colonize the epicuticular wax layer of pome fruit, including apple (*Malus* x *domestica* Borkh.) (Brown and Sutton 1995; Johnson et al. 1997; Williamson and Sutton 2000; Díaz-Arias et al.

2010). While SBFS has been studied in North America for well over a century (e.g., Hickey 1960; Williamson and Sutton 2000; Brown and Sutton 1995; Batzer et al. 2005; Cooley et al. 2007), its etiology and epidemiology elsewhere in the world have just begun to be explored (Grabowski and Wrona 2004; Grabowski 2007; Späth and Mayr 2008; Spolti 2009). The common names “sooty blotch” and “flyspeck” arose from recognition of two general morphological types of colonies on apple fruit. Colonies of the “sooty blotch” type form olive green to black mycelial mats, whereas “flyspeck” denotes clusters of a few to several dozen small (1–4 mm diameter), slightly raised, black, shiny dots (Williamson and Sutton 2000). Additional speck morphologies on apple include “compact speck” and “discrete speck” that have smaller clusters of dots more densely arranged (Batzer et al. 2005).

In North America and Europe, SBFS is an increasing threat to profitable apple production due to recent restrictions on the use of highly effective fungicides and the increased hectarage of organic apples (Merwin et al. 1994; Williamson and Sutton 2000; Späth and Mayr 2008). The presence of SBFS on the apple surface lowers quality and market value of the fruit because the fungal colonies cause an unacceptable appearance of the fruit to consumers (Williamson and Sutton 2000). Economic losses as high as 90%, because of downgrading fruit from fresh-market to processing use, have been reported in the eastern United States, where SBFS is a major economic problem (Williamson and Sutton 2000). The risk of rejection of fresh fruit by consumers depends on the proportion of the fruit covered with SBFS blemishes (Yue et al. 2007).

SBFS can be suppressed by the use of fungicide sprays during fruit maturation (Rosenberger et al. 1996). Nevertheless, poor fungicide coverage, inadequate pruning and environmental conditions highly favourable for disease development contribute to sporadic control failures (Cooley et al. 1997). To compensate for inadequate field control, postharvest removal of SBFS on apples is sometimes attempted by disinfestant dip and brushing treatments (Hendrix 1991); however, efficacy of these treatments differed depending on the method used and the SBFS species present (Batzer et al. 2002).

The availability of reliable and cost-effective assessment methods is critical to assessing the value of treatments in reducing the economic impact of many diseases (Nutter et al. 2006; Madden et al. 2007), including SBFS (Batzer et al. 2002). In general, intensity of diseases that affect fruit can be measured as incidence (proportion of affected fruit in a sample), severity (percent of the fruit surface affected), or number of lesions or colonies (Madden et al. 2007). Measuring only SBFS incidence may be of limited value because it does not take into account disease intensity, especially for situations such as SBFS infestations in which the size, location, and visibility—and therefore economic impact—of individual fungal colonies can vary greatly. In conducting visual estimations of disease severity, standard area diagrams (SADs) can enhance accuracy and precision of estimates, especially by inexperienced raters (Nutter and Littrell 1998). The usefulness of SADs in increasing both accuracy and precision of severity estimates can be measured by statistical methods (Nutter et al. 2006; Madden et al. 2007) that can help to identify needs for further adjustments of the scale and provide continuous training of raters to correct bias (Nutter and Schultz 1995).

Development of SBFS signs has been estimated in terms of incidence (Hendrix 1991), severity (Batzer et al. 2002), number of colonies per apple (Batzer et al. 2002) or a severity index (Mayr and Späth 2008) for evaluating effectiveness of field or post-harvest control treatments. Batzer et al. (2002) described and compared several assessment methods to evaluate efficacy of post-harvest dip treatments for SBFS removal. Initially, severity values were visually estimated and converted into an ordinal scale encompassing six classes (0 to 6). In order to reduce data variability, a second experiment utilized a SAD of SBFS severity that improved accuracy of assessments by the same rater and provided direct assessment of percent severity rather than reliance on general classes of severity (Batzer et al. 2002).

Although a SAD has already shown value in assessing SBFS severity (Batzer et al. 2002) several limitations must be overcome to allow its broader use in epidemiological and disease control studies. First, the scale was developed to represent severity only on the lateral fruit view, whereas the disease often occurs at the stem end of the fruit (Spolti 2009). Second, the diagrams were not published and consequently are not widely available. Third, formal statistical assessment was not performed to quantify the gain in accuracy and precision in comparison to alternative methods.

Therefore, the objectives of this work were to 1) develop a new standard area diagram to aid visual SBFS severity estimations on both the peduncle and lateral views of apple fruit; and 2) quantify impact of the scale on accuracy and precision of disease estimations by raters.

Materials and methods

Image acquisition and analysis

Mature apple fruit (cvs. 'Mutsu' and 'Fuji') exhibiting SBFS signs were obtained from orchards in Brazil, located in the municipalities of São Joaquim and Fraiburgo in Santa Catarina State and Vacaria in Rio Grande do Sul State. A total of 364 'Fuji' and 81 'Mutsu' fruit were photographed from two perspectives—from the top (peduncle region) and laterally—using a digital camera (Sony DSC-H50, 9.1 megapixels). The peduncle view was added to earlier methods (Batzer et al. 2002) because several fruit showed signs only or predominantly at the peduncle region.

A preliminary visual analysis of the images was performed to arbitrarily select 160 images representing an apparent gradient in disease severity. For the selected images, "true" disease severity was estimated using ASSESS 2.0 image software (Image Analysis Software for Plant Disease Quantification, St. Paul, MN, USA, APS, 2008). Severity of SBFS was defined as the proportion of the apple cuticle covered by the signs of the disease. For colonies consisting of speck-like signs, diseased area was defined as the total tissue portion within the limits of clustered sclerotium-like bodies (Batzer et al. 2005) collectively constituting a single colony. Prior to severity estimation, single colonies were painted manually in blue using a computer tool to facilitate discrimination by the software. Images were 1,900×1,800 pixels in size.

SAD construction and validation

The methodology for constructing the new SAD differed from a previous report (Batzer et al. 2002) in several aspects. In that study, apples showing very low severity (<2%) were not used. In our study, lower and upper bounds of the scale interval, number of diseased fruits and associated severity levels of the revised SAD were defined taking into consideration the frequency of occurrence of severity values encountered in the survey. While the previous diagram (Batzer et al. 2002) targeted only the lateral view, we constructed a dual-view scale, for the top as well as the lateral view.

The new SAD set was validated by eight raters who independently assessed percent severity in two series of selected images: 28 images representing the lateral view and 20 the top view. Each image series included a range of severity levels on fruit that exhibited signs typical of sooty blotch mycelial morphology, speck morphology, or both. Each fruit image was randomly displayed on a computer screen to each rater for 30 s using Power Point™. Two consecutive assessments were made by a rater. First, visual severity (%) assessment was made without any aid; second, the same fruits were displayed in the same order and severity was again assessed by the rater with the aid of the SAD. In the second case, photo paper depicting the SAD (two series of fruits in gray tones and signs painted in black color) with corresponding SBFS severity for each fruit view was given to the rater, who used them as a reference to estimate severity during a 30 s period per image.

Data analysis

Descriptive statistics were used to characterize severity distribution in the sample of digital images. Reproducibility of the estimations was assessed by a pair-wise comparison among raters using correlation analysis. Correlation concordance coefficient (ρ_c) analysis (Lin's CCC) (Lin 1989) was employed to measure agreement (both precision and accuracy) between estimated and actual SBFS severity values with or without the aid of the SAD (Nita et al. 2003; Madden et al. 2007; Bock et al. 2008). Scatter plots were used to characterize CCC components (C_b = bias estimation factor, ν = scale shift indicating changes in line slope, and u= location shift indicating changes in line height, describing accuracy; and R = correlation coefficient, describing precision) for each rater. In addition to concordance analysis, linear regression was conducted for the overall relationship between actual and estimated severity values of pooled raters` estimations. Intercept (a) and slope (b) values for estimations with and without the scale aid were compared with t-test to evaluate the null hypothesis

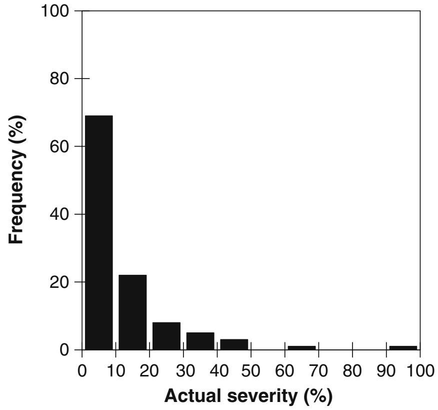

Fig. 1 Frequency of sooty blotch and flyspeck severity (% area diseased) in 160 images of 'Fuji' and 'Mutsu' apple cultivars showing typical symptoms on the top or the lateral view of apples

that $a=0$ and $b=1$ ($P<0.05$) (Nutter and Schultz 1995; Bock et al. 2009). Statistical analysis and graphs were performed in SigmaPlot (SPW7, SPSS Inc., Chicago, IL).

Results

SBFS severity ranged from 0.4% to 98%; most fruit had <10% severity and only one fruit had severity >65% (Fig. 1). Distinct patterns of SBFS signs were observed depending on the fruit view. At the top view, signs were aggregated around the peduncle, especially for apples with SBFS <15%.

The diagrammatic scale developed for the lateral view shows ten severity levels in exponential increments that range from 0.4 to 65% severity (Fig. 2). For the top view, eight levels were defined with actual severity ranging from 1 to 63% (Fig. 2). Coalescence of SBFS colonies was commonly observed in fruit showing severity >3%, so coalescence was represented in the diagrammatic scale at severity levels exceeding that value (Fig. 2).

Accuracy and precision of severity estimates improved substantially when using the SAD as an assessment aid; concordance correlation coefficient (ρ_c) values increased from 0.79 to 0.95 with larger increase for assessments in the lateral view of the fruits (Fig. 3).

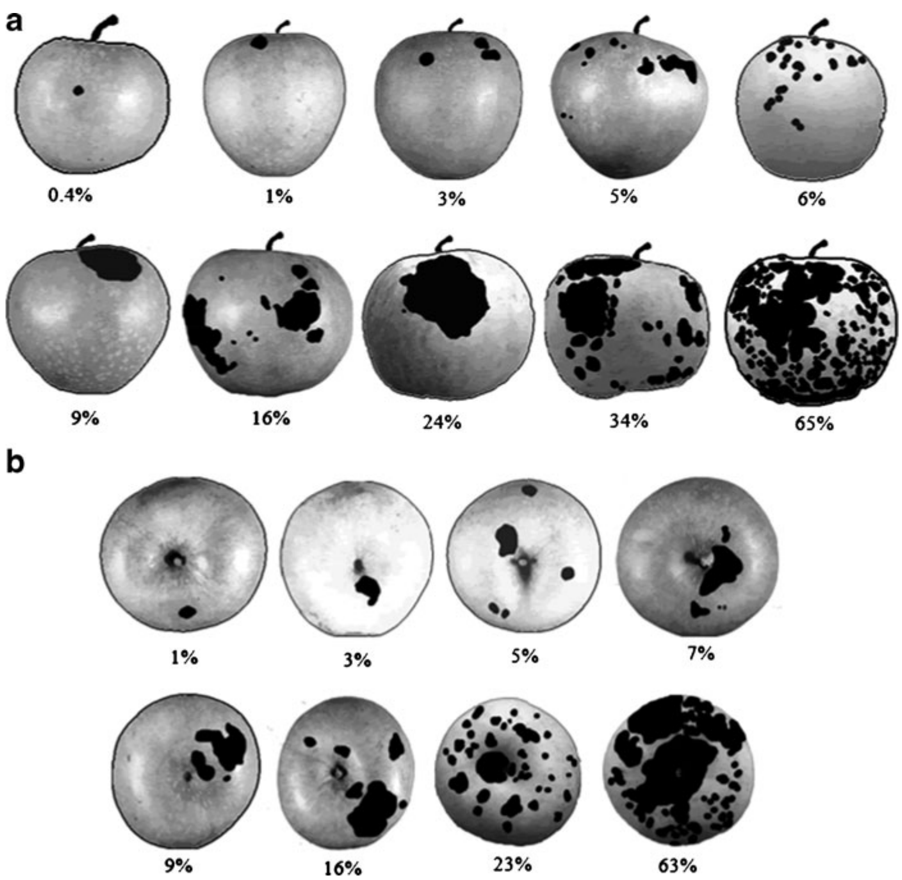

Fig. 2 Standard area diagram sooty blotch and flyspeck severity (% area diseased) in two fruit views: the lateral **a** and top view **b**

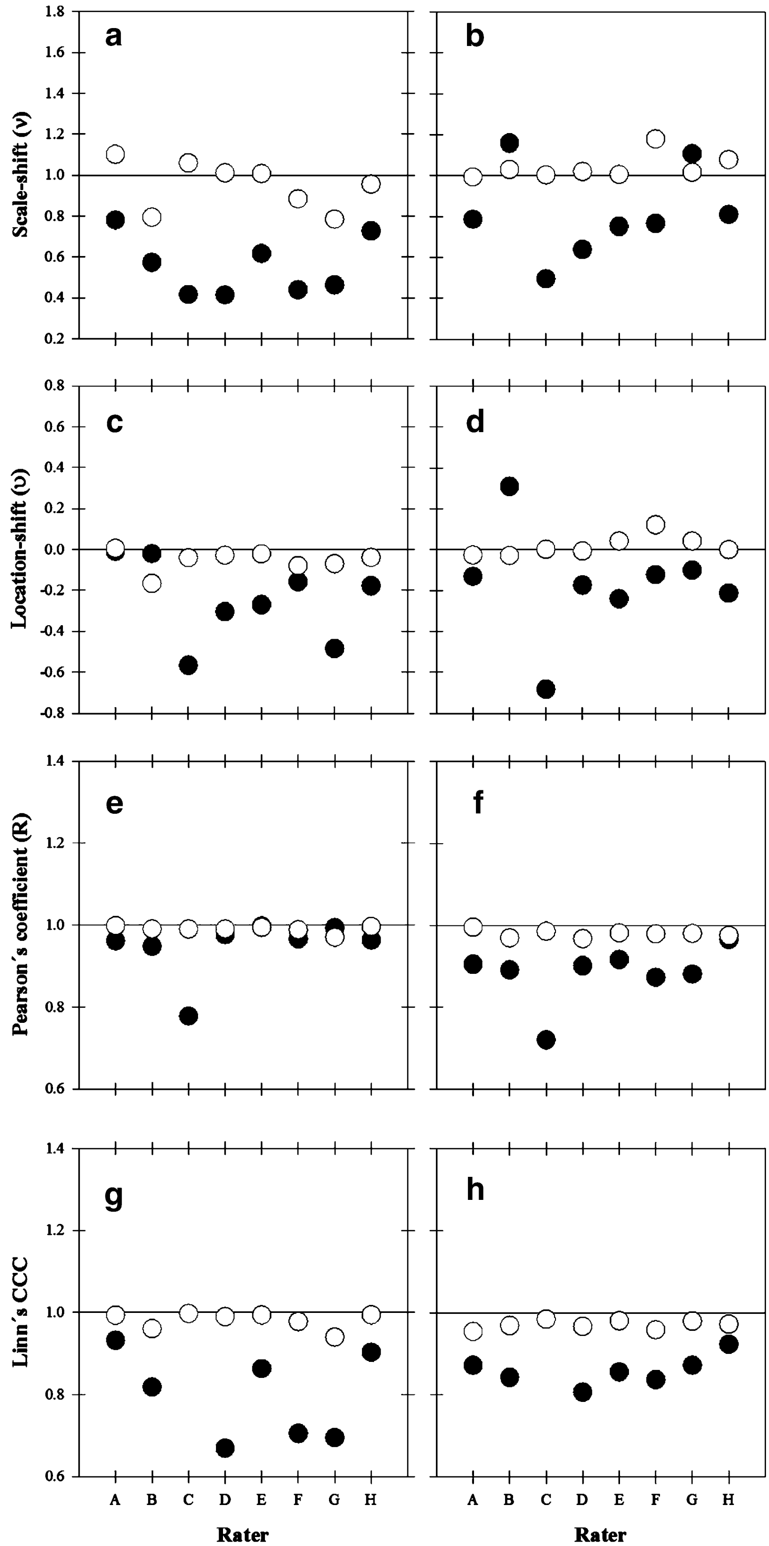

Fig. 3 Scale-shift **a**, **b**, location-shift **c**, **d**, Pearson's coefficient **e**, **f** and the concordance correlation coefficient (Lin´s CCC = ρ_c) **g**, **h** analysis in relation to accuracy of estimation by eight raters who assessed sooty blotch and flyspeck severity of apples with (*white circle*) or without (*black circle*) a standard area diagram as assessment aid for the top view **a**, **c**, **e**, **g** or lateral view **b**, **d**, **f**, **h** of the fruit. Perfect agreement, occurs when the scale-shift (ν)=1, location-shift (u)=0, Person´s coefficient (precision)=1 and Lin´s CCC (ρ_c)=1

When CCC parameters were evaluated separately for each fruit view, an overall tendency to underestimate severity was observed for most raters, especially for the top view and without the assessment aid (Fig. 3). However, when raters used the assessment aid, fruit view did not influence ρ_c values, probably because precision of estimation was not affected (Table 1).

The use of the SAD also improved reproducibility of the estimates among raters. Pearson correlation coefficients (r) values for estimates made with the SAD aid ranged from 0.84 to 0.96 compared to 0.61 to 0.91 without the assessment aid (Table 2). The overall ρ_c increased from 0.81 to 0.95 when the SAD was used to aid visual estimation (Fig. 4). In the regression analysis for the pooled values between actual and estimated severity without the SAD aid, intercept and slope were significantly different from zero and 1, respectively ($P<0.001$). When using the assessment aid, intercept was not different from zero ($P>0.05$) whereas slope was statistically different than 1 ($P=0.03$) (Fig. 4). When the raters' estimates were pooled, an overall tendency to overestimate SBFS severity was observed, especially for severity values <10% (Fig. 4). For severity values >35% the tendency was to underestimate severity mainly for the top view of the fruit.

Discussion

In southern Brazil, SBFS severity levels, although lower than those reported in the eastern USA (Brown and Sutton 1995; Sutton et al. 2005), can exceed 50% when no fungicides are applied (Spolti 2009). In orchards utilizing IPM-based fungicide spray programs, however, most SBFS-infested apples had severity values of <10%. In these orchards, another difference compared to what is commonly reported in North America was that about 75% of infested fruit exhibited signs predominantly around the peduncle region (Spolti 2009). Fungicide sprays sometimes fail to protect the stem cavity region of the fruit because they may not penetrate this zone. Moreover, wetness duration may be longer in this region compared to the lateral face of the fruit (Trapman 2004). Focusing on SBFS severity in the peduncle region, as we have done, may have practical value for assessing the effect of thinning (which reduces fruit clustering and enhances spray access to the peduncle region, besides reducing wetness duration) pruning (Cooley et al. 1997), increasing spray volume (which can improve spray coverage of fruit) (Sutton and Walgenbach 1998) or the impact of SBFS warning systems (Brown and Sutton 1995; Duttweiler et al. 2008).

Most SADs have targeted foliar diseases (Vereijssen et al. 2003; Godoy et al. 2006; Bock et al. 2009); relatively few have focused on fruit (Batzer et al. 2002; Spósito et al. 2004). In general, a tendency to overestimate actual disease severity has been detected in most SAD validation studies, especially for low levels of severity (Forbes and Korva 1994; Spósito et al. 2004; Bock et al. 2008; Bock et al. 2009). In our study, however, we observed a slight tendency to underestimate disease severity, especially for medium to high severity levels even when using the assessment

Table 1 Mean and confidence interval (α=95% CI) for components of the concordance correlation coefficient (Lin's CCC = ρ_c) analysis (R, C_b, ν, u) that takes into account both accuracy and precision of the visual estimates of sooty blotch and flyspeck symptoms on individual or pooled views of the fruit

Fruit view	Diagram aid	N	Pearson`s R	ν	u	C_b	ρ_c	Upper 95% Z CI	Lower 95% Z CI
Top	Yes	20	0.99	0.950	-0.056	0.989	0.981	0.990	0.960
Top	No	20	0.87	0.554	-0.249	0.799	0.760	0.860	0.650
Lateral	Yes	28	0.97	1.040	0.018	0.996	0.971	0.980	0.960
Lateral	No	28	0.82	0.814	-0.169	0.914	0.811	0.910	0.710
Both	Yes	48	0.97	1.136	0.113	0.986	0.950	0.980	0.900
Both	No	48	0.82	0.860	0.052	0.987	0.798	0.890	0.700

N = total number of samples (fruit images for all raters); in using Lin´s CCC (ρ_c) for agreement, R = Pearson`s correlation coefficient which measures precision, ν = scale shift (slope); u = location shift (line elevation), C = is the bias correction factor indicating accuracy and CI = the confidence interval for Lin´s CCC (Lin 1989)

Table 2 Reproducibility matrix and Pearson`s correlation coefficient (*R*) for pair-wise comparisons among raters when assessing sooty blotch and flyspeck severity on apple fruits with or without a standard area diagram as an assessment aid

Raters		Standard area diagram aid							
		A	B	C	D	E	F	G	H
No assessment aid	A	–	0.84	0.96	0.97	0.96	0.93	0.94	0.94
	B	0.85	–	0.96	0.93	0.96	0.92	0.97	0.96
	C	0.62	0.74	–	0.97	0.98	0.95	0.96	0.98
	D	0.81	0.83	0.73	–	0.96	0.94	0.94	0.96
	E	0.69	0.82	0.68	0.70	–	0.95	0.95	0.97
	F	0.90	0.88	0.61	0.84	0.72	–	0.93	0.95
	G	0.90	0.81	0.68	0.91	0.61	0.88	–	0.95
	H	0.88	0.86	0.91	0.84	0.83	0.85	0.85	–

aid. Likely explanations may relate to the fact that speck-type colonies are not visually continuous, but instead formed by clusters of small specks that constitute larger colonies (Williamson and Sutton 2000). In addition, the varied shades of the sooty blotch colonies, from light gray to black, may introduce error in visually estimating this kind of sign especially during the initial stages of SBFS colony appearance (Sutton 1990). Finally, the relatively larger interval for the higher severity levels of the diagram compared to the lower levels may have also contributed to error. Previous work has demonstrated that a linear increase in severity values in SADs may provide higher accuracy in the rater's estimates than logarith-

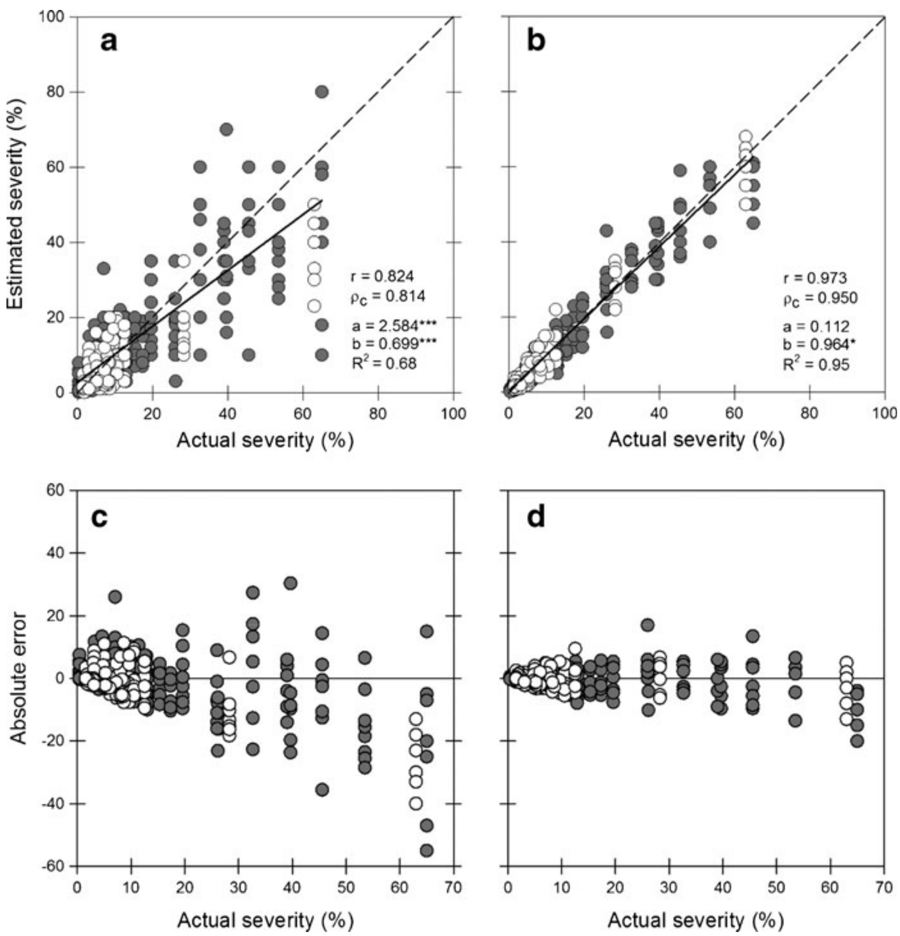

Fig. 4 Scatter plot and linear regression between actual (measured by image analysis) and visually estimated severity of sooty blotch and flyspeck on apple fruits by eight raters and absolute error (estimated minus actual severity). Assessments for top view (*white circle*) or lateral view (*gray circle*) were made unaided **a**, **c** or using a standard area diagram as assessment aid **b**, **d**. Dashed line represents the 1:1 line. *** designates statistical significance level of $P<0.001$ and * designates statistical significance level of $P<0.05$ when assessing whether a (intercept)=1 and b (slope)=0 in the linear regression analysis. R = Pearson`s coefficient, a measurement of precision in Lin´s CCC (Lin, 1989). $\rho_c$ (Lin`s CCC) = Concordance Correlation Coefficient (CCC = R × C_b)

mic increase (Nutter and Esker 2006). The smaller intervals defined in the severity range of 0.4–9% in our scale was mainly due to the observed higher frequency of severity values in this range in our natural infected samples; in this range the increase was linear. However, an exponential increase is verified when considering all the severity values depicted in our SAD.

Nevertheless, our work strengthened the credibility of the assessment method proposed by Batzer et al. (2002) by validating it in a rigorous manner. We demonstrated that both accuracy and precision of SBFS severity estimates increase is vindicated when a rater used the SAD as an assessment aid. It could be particularly helpful for training inexperienced raters who demonstrate a tendency to underestimate disease severity. Reproducibility among raters increased considerably when they used the assessment aid. Batzer et al. (2002) observed a significant difference among raters in the first experiment, so a single rater was used in the second experiment to decrease variability. Finally, we broadened the approach by adding a peduncle-end assessment, which is of particularly relevance to observations of SBFS sign distribution in Brazilian apple orchards.

The difficulties associated with the measurement of actual disease severity in 3-D surfaces (fruits) compared to 2-D surfaces (leaves) have led some authors to propose specific and complex methods to model and estimate 3-D area surfaces covered by disease symptoms or signs. For instance, Corkidi et al. (2006) proposed the use of a special digital capture and analysis of rotating diseased (*Colletotrichum gloesporioides*) mango fruits for the estimate of actual severity. Our SAD is proposed as an aid to provide simple, quick and accurate visual estimate of severity in specific views of the fruit and is not intended to provide overall severity estimation on a 3-D surface.

Our revised SAD to aid SBFS severity assessment has proven to be a reliable assessment method that may allow acceptably accurate quantification of the benefits of treatments in reducing SBFS and direct comparison among studies on varietal resistance, disease progress curves or fungicide control efficacy that takes severity into account. In the past, the lack of a uniform, verified method has made these tasks difficult. The further test and validation of our revised SBFS standard area diagram in additional trials will add to its credibility as a standard tool for SBFS research as well as quality control activities such as fruit sorting in packing houses.

Acknowledgements The authors thank the Programa de Pós-graduação em Fitotecnia (UFRGS) and CAPES—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for providing a scholarship to the first author.

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