Research Article

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Digital phenotyping of coconut and morphological traits associated with eriophyid mite infestation

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Abstract

Observations were recorded on traits associated with mite infestation related at two stages of button on six different coconut cultivars over three years. Highly significant correlation was found between mite damage score with color or weight of tepal. Step-wise multiple regression of the data analysis showed color of inner tepal as major trait associated with infestation by eriophyid mite. Other traits are ratio of tepal weight to tepal area, per cent of buttons with pink discoloration or with resin, tepals of regular aestivation and gap between fruit and tepal. Digital phenotype data of 83 image files were used to calculate color signature and correlated the same to mite damage score over three years. Red spectral values were found to vary from 14 to 251, green values to 12 to 237 and blue to vary from 5 to 183. Spectral values red max, green max, 3* Red + Green max had high significant negative correlation (>-0.4) with mite damage. Color and firmness of fruits and tepals of three coconut varieties were further analyzed where, fruits and tepals of COD variety showed high red/green (a* value of Hunterlab) >12. Firmness of 3 month old tepal and fruit of Benualim (BGRT) tall variety was (penetrometer reading >38) higher than other varieties.

Keywords: Colour, Hunterlab, palms, penetrometer, spectral, tepal

Introduction

Eriophyid mite (*Aceria guerreronis* Keifer) (Eriophyidae: Acari) is originaly a pest of coconuts grown in Jamaica, Mexico and other Latin American and Caribbean countries. The pest has later spread to African countries and it became a serious pest of coconut. It had further spread to few Asian countries in late 1990s. The incidence of the pest was reported for the first time in Kerala, India in 1998 (Sathiamma *et al*., 1998). Coconut palm suffers serious economic loss due to the mites feeding the fruit just below tepals or (most commonly, but erroneously referred to as bracts). Host plant resistance is an important and effective way of managing the pest under the IPM strategies. High degree of mite tolerance was seen in a Cambodian variety with tight tepals (Mariau, 1986). Biophysical traits of microhabitat of the pest in the plant provide an indirect means to select resistance to mite. Among the traits analyzed, shape (Julia and Mariau, 1979; Mariau, 1986; Moore and Alexander, 1990) colour of the fruit (Moore and Alexander, 1990), tightness of tepals (Howard and Rodriguez, 1991), gap between the rim of the fruit (Aratchige *et al*., 2007), aestivation of tepals

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(Lawson-Balagbo *et al*., 2007) were found to be associated with mite resistance. Moore (1986) found two types of bract (perianth or tepal) arrangement on coconuts that offer different degrees of adpression. The degree of adpression decreases with increasing size of the nut (Julia and Mariau, 1979; Mariau, 1986; Otterbein, 1988). Surface of the damaged nuts exude resin, which aids to trap and kill the mites (Moore and Alexander, 1987). Digital phenotyping of color using image files of the fruit or vegetable is an emerging tool to study the variation in color. Color of fruit or tepal is important trait associated with resistance to the pest. Orange colored cultivar Chowghat Orange Dwarf (COD) display low pest damage among the cultivars screened by many investigators. Measurement of color has wide applications such as canopy reflectance to detect fungal disease in oil palm (Lelong *et al*., 2010). Digital phenotyping is emerging as tool to quantify plant traits like fruit color of tomato (Darrigues *et al*., 2008) and leaf phyllometry in tea (Mugnai *et al*., 2008).

Objectives of present study were (i) to find relationship between the plant biophysical traits to the resistance to eriophyid mite in six cultivars observed over three years at two stages of fruit growth using step-wise multiple regression approach. (ii) to analyze the primary and secondary colors derived from red, blue and green spectra in the images of fruit and outer tepal of the six cultivars of coconut and its relationship with damage to eriophyid mite. (iii) to quantify the color and firmness of coconut tepal and fruit using Hunterlab instrument and penetrometer respectively.

Materials and methods

Observations were recorded at three plots each with six palms of six coconut cultivars planted in experimental fields of Central Plantation Crops Research Institute, Kidu, Dakshina Kannada district, Karnataka, located at 12° 45’ N latitude and 75° 32’ E longitude at an elevation of 110 m above MSL. The mean temperature ranges from 20 °C to 32 °C and the mean annual rainfall is 4000 mm. Major rainfall is restricted to June to September months. Six coconut cultivars used in this study were Chowghat Orange Dwarf (COD), Gangabondam Dwarf (GBD), Gudanjali Dwarf (GDD), Malayan Yellow Dwarf (MYD), West Coast Tall (WCT) and Andaman Ordinary Tall (ADOT). Observations were recorded during three years (2003, 2004 and 2005) in two seasons pre-monsoon (March-April) and post-monsoon (November-December). Two and three month old buttons (young fruits) formed the study material. A sample of five buttons used for recording the observations and data analysis of 21 variables from 387 sets. Color of tepal, petiole, inflorescence and button was described from yellow to orange, green or brown and a scale of 0 to 10 was assigned based on the intensity (Table 1). Vertical and horizontal circumferences of each of the buttons were measured. Vertical circumference with and without tepals was also measured. Cap space (gap between fruit and tepal) was worked out by subtracting the vertical circumference without tepals from vertical circumference with tepals. Shape index was worked out by dividing the horizontal circumference by vertical circumference. Weight of the whole button and one inner tepal each was measured in grams (g) and length and width of one tepal were measured and expressed in centimeters (cm). Arrangement of inner tepals was observed and classified as contortion/regular twisting and imbricate/irregular twisting based on overlapping of tepals. Percentage of buttons having regular arrangement was worked out from this data. Coconut fruits exude a gum like resin as a defense mechanism when infested by the mite. The number of buttons producing resin and the percentage of buttons with resin was worked out. Few coconut buttons/fruits developed pinkish discoloration when infested with mites as a defense reaction. The number of buttons showing pinkish discoloration in each case was also

Table 1. Color descriptions and scale of tepal, petiole, inflorescence and button

Color	Scale
Light yellow	0.5
Yellow	1
Light orange	2
Orange	3
Light green	4
Green	5
Yellowish green	6
Light brownish green	7
Light brown	8
Brownish green	9
Brown	10

counted and the percentage of buttons with pink coloration was calculate. Few more traits from these preliminary observations were also derived and given below:

Tepal area (cm^2) = tepal length (cm) x tepal width (cm)

Specific tepal weight (g cm^{-2}) = tepal weight (g) / tepal area (cm^2)

Tepal to button weight ratio = weight of tepal (g) / weight of the button (g)

HC-3teplen = (horizontal circumference (cm)) – 3*(length of tepal (cm))

VC-3 tepwid = (vertical circumference (cm)) – 3*(width of tepal (cm))

Damage due to mite infestation was scored on 0 to 4 scale as per standard procedure:

0 : free from damage; 1: 1-10 % damage; 2: 11-24 % damage; 3: 25-50 % damage and 4: >50 % damage.

Mite damage index was calculated using the following formula:

Index = (No. of nuts/buttons with 0 score) x 0 + (No. of nuts/buttons with 1 score) x 1 + (No. of nuts/buttons with 2 score) x 2 + (No. of nuts/buttons with 3 score) x 3 + (No. of nuts/buttons with 4 score) x 4/ (Total number of buttons/nuts).

SPSS 15.0 software was used for analyzing correlation of each trait to mite damage score and a step-wise regression for relating multiple traits to mite damage score. Stepwise regression is a semi-automated process of building a model by successively adding or removing variables based solely on the *t*-statistics of their estimated coefficients. It is especially useful for choosing among a large numbers of potential independent variables so as to fine-tune a model to most predictive.

Using a digital camera, pictures of fruit along with tepal kept at 30 cm distance and at uniform lighting were captured. These images were analyzed using Adobe Photoshop and the variation in red, blue and green spectra as seen by maximal and minimal values were recorded. Damage due to eriophyid mite observations were recorded for three years (2003, 2004 and 2005) during pre-monsoon and post-monsoon periods. A sample size of 3 palms in each cultivar was used and two images of each sample were taken for the mean values. Eighty three image files of fruits and tepals of 3 and 5 month old six coconut cultivars were used as materials. Damage score due to mite incidence were recorded over three years and the mean value was worked out. Based on spectral values of red, blue and green in the images, yellow spectral value was worked out using the formula yellow = green-blue. Other secondary colors like orange, violet, brown *etc*. were calculated using the color wheel concept orange = red +yellow, violet = red+blue, brown = red+green. Digital phenotype data was used to calculate color signature as 3*red+green and correlated all the spectral values thus obtained to the mite damage score. Correlation was worked out between each of spectral values and mite damage score. A statistical t-test was performed to check the significance of correlation.

Hunterlab instrument (tri-stimulus colorimeter) was used to measure the color as brightness (L*), red/green ratio (a*) and blue/yellow ratio (b*) values of the fruit and tepal samples in the two and three months old buttons of coconut in five palms each of three varieties COD, MYD and Benaulim. These three varieties were chosen to know the specific role of color (orange, yellow and green) of fruit in three varieties. Fruits of three varieties chosen are round in shape and hence uniform for shape. Penetrometer instrument was used to measure the pressure required to penetrate the fruit and tepal samples in the two and three months old buttons of coconut in five palm of three varieties COD, MYD and Benaulim.

Results and discussion

Coconut gardens studied had severe incidence of mite infestation. Despite heavy incidence of the pest, some of the palms remained free from mite infestation. Some palms showed high degree of susceptibility. Thus, we used this pest hot-spot area and performed the study to look for mite resistance. Correlation of individual traits to mite damage score was found to be significant (p=0.05) for most traits studied *viz*., percentage of regular tepals, percentage of fruits/buttons with resin, ratio of weight of tepal to area of tepal, ratio of weight of tepal to weight of button, weight of tepal (g), color of tepal, color of petiole, color of nut and percentage of pinkish discolored buttons (Table 2). Cap space was not found significantly correlated to mite damage score.

Table 2. correlation between individual traits with mite damage score and goodness of fitness of regression equation

Trait	Correlation coefficient
Percentage of regular tepals	-0.135
Percentage of fruits/buttons with resin	0.103
Ratio of weight of tepal to area of tepal	0.321 **
Ratio of weight of tepal to weight of button	0.291 **
Weight of tepal (g)	0.340 **
Color of tepal	0.386 **
Color of petiole	0.130
Color of nut	0.139
Percentage of pinkish discoloured buttons	0.228 *

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	0.12224	3.90986	1.43265	0.657666	387

*Significant at 5% level, **significant at 1% level

Tepal color, specific tepal weight, tepal aestivation and defense mechanisms of pink coloration or resin production, colour of petiole, and the gap between fruit and tepal were found to be important traits contributing to mite resistance in coconut palm. These traits together had shown high degree of association (R^2 value = 0.312) and also displayed good fit of (Table 2) predicted *vs* observed values. The final regression equation worked out as follows,

Mite damage index score = 0.262 + 0.436 (color tepal) + 0.294 (specific tepal weight) + 0.133 (per cent pink) + 0.155 (per cent resin) – 0.133 (petiole color) – 0.085 (per cent regular) – 0.085 (cap space).

Of the seven traits associated with resistance, three are color related traits, such as colour of tepal, color of petiole and pinkish discoloration. Tepal forms the microhabitat of the eriophyid mite. Color of the tepal is significantly (r=0.386) related to resistance to eriophyid mite. Colour displays serve to render invertebrate herbivores (*i.e.*, parasites) more conspicuous to predators (Blount and McGraw, 2008). There are no reports on association of color of tepal to mite resistance in coconut. We emphasize the importance of colour of tepal as an additional important trait being a habitat of the pest. There are two contrasting reports on color of fruits relating to damage due to mite. Orange colored fruits display minimal mite damage (Varadarajan and David, 2003) and green colored fruits show low infestation of mite (Moore and Alexander, 1990). Variations for traits under study among the coconut varieties are given as Table 3. The conclusions drawn could vary due to the type of material used in the study and variation examined. Dwarf cultivars due to self pollination have uniformity in color of plant parts such as petiole, tepal, fruit and inflorescence in the same plant. Tall cultivars show differences or uniformity among plant parts. Leaf petiole color shows association with resistance to mite. It is a heritable trait in coconut controlled by (R, G) two dominant genes (Bourdeix, 1999). Present study finds color of tepal and petiole alone as related to mite resistance. Direction and intensity of changes in eriophyoid-infested plant organs depend on mite genotype, density, or the feeding period, and are strongly differentiated relative to host plant species, cultivar, age and location (Petanovic and Kielkiewicz, 2010). Pinkish discoloration is found in a few samples after infestation by mite. Percentage of pinkish discolored buttons was worked out to know its association with pest resistance. Defense indication by pink discoloration is one of the five potential visual functions against herbivory (Lev-Yadun and Gould, 2007). Transcript levels of genes related to anthocyanin production were up-regulated

Table 3. Mean values of the traits of coconut in different varieties

Variety	Cap space	Shape index	% regular	% resin	tepal wt/area	% pink	Color petiole	Color tepal	Tepal wt/area	Color nuts	W ttep/ butt	Tepal weight	Damage index
COD	1.79	0.88	0.12	0.12	0.99	0.05	3.00	2.58	0.99	2.97	0.24	5.45	1.01
MYD	1.99	0.88	0.27	0.01	0.92	0.01	1.00	0.97	0.92	1.00	0.20	5.18	1.23
WCT	1.87	0.91	0.13	0.06	1.09	0.03	5.00	8.30	1.09	5.05	0.24	8.27	2.51
GBGD	1.78	0.84	0.24	0.14	0.71	0.00	5.00	4.27	0.71	4.98	0.14	4.39	1.09
GDD	1.65	0.90	0.20	0.12	0.73	0.05	5.00	4.39	0.73	5.00	0.14	4.63	1.58
CD 5%	0.06	0.01	0.03	0.02	0.04	0.01	0.02	0.30	0.04	0.16	0.01	0.25	0.14

just after 4 days of mite infestation in tomato (Kant *et al*., 2004). Strait Settlements Apricot cultivar with a pinkish colored fruit showed low damage due to mite at experimental fields (data not shown) and the role of anthocyanins in offering protection of plants against mites.

This study highlights the use of seven traits to get clear picture which include the few traits used by earlier workers such as color and tepal traits. Quantification of color of tepal and petiole using appropriate techniques will add to further knowledge on the mechanism of coconut plant resistance to mite.

Coconut fruits exude a gum like resin as a defense mechanism when infested with mite that trap and kill the mites (Moore and Alexander, 1987). The plant exuding resins in all the affected fruits are able to better defend it from the damage due to the pest. Percentage of resin exuding buttons thus gives a clear association with pest resistance. Sesquiterpene hydrocarbons in the hexane extract of the resin of *Commiphora holtziana* exhibited a repellent effect on ticks and mites (Birkett *et al*., 2008). Monoterpenes present in the resins are known as a primary defense mechanism to protect the pine trees against bark beetle infestation (Thoss and Byers, 2006).

Significant correlation was noticed between the roundness of coconuts and damage by the coconut mite as the rounded morphology of nuts creates tighter adpression of the perianth onto the nut surface, restricting the entry of the coconut mite beneath the perianth and its ability to establish a colony on the nut (Mariau, 1977; Moore and Alexander, 1990; Howard and Rodriguez, 1991). Aestivation of inner tepals in coconut are of two types *viz*., contortion (with regular twisting or overlapping of tepals at one end) and imbricate (with irregular twisting or non-overlapping of inner tepals or one tepal overlapping at both ends). The percentage of flowers bearing corolla with contortion arrangement is varietal character and varies from 10 per cent (dwarfs) to 27 per cent in (talls). Mite population is positively correlated to tepal area (Varadarajan and David, 2002). Specific tepal weight as measured by ratio of tepal weight per tepal area decides the toughness of the tepal tissue. Coconut buttons with high values of specific tepal weight are tough tissues and hence reduced feeding and development of mites beneath them.

Tightness of tepals also provides resistance to mite (Howard and Rodriguez, 1991) as the gap between fruit and tepals decides the entry of mite and their predators (Aratchige *et al*., 2007). Gap between the fruit and the tepal is predicted by the ratio of length of fruit to radius of tepal (Varadarajan and David, 2002). Large gap facilitate the entry of predatory mites and hence the habitat is not congenial for herbivorous mite. Gap between fruit and tepal is also accurately measured by microscopy. There is significant difference in this gap space in uninfested fruits between three varieties Sri Lankan Green Dwarf, Sri Lankan Tall and their hybrid (Aratchige *et al*., 2007). Sri Lankan Green Dwarf has become susceptible to mites as the fruits of this variety are small sized with an elongate shape. Hence the gap in the fruits of this variety before infestation is large enough for the eriophyid mite to enter but small for the predatory mite. However, this perianth fruit rim gap in infested fruits do not differ significantly and hence accessible to predatory mites. Access to predatory mites long after the eriophyid mite reach sufficient population is insufficient to keep the pest populations below normal levels. Hence, the measurement or prediction of gap in uninfected fruits is important and need breeder’s attention.

Aestivation also decides the tightness of the tepals (Moore, 1986) and consequently the population ratio of herbivorous and predatory mites (Lawson-Balagbo *et al*., 2007). In case of irregular twisting or imbricate aestivation, adherence of perianth or tepal is not tight, and also permits the entry of mite into nuts reaching maximal population (Paul and Mathew, 2003). But the nuts possessing the regular twisting of tepals/contortion arrangement can offer tight adherence of tepals with nuts hence not allowing the entry of mites into the nut (Moore, 1986). We report the percentage of buttons with regular aestivation to negatively correlate (-0.135) to the damage by mite. Percentage of regular aestivation trait varies among varieties. Variation is present in coconut cultivars for this trait and Tall varieties possess higher percentage of contorting tepals than dwarf varieties whereas Dwarf x Tall hybrids are intermediate (Davis *et al*., 1990). There are few reports on combination of two or three traits to pest resistance. Orange colored nuts, small sized inner tepals (radius less than 2 cm) arranged with cleft angle >136° are found to have fewer population of mites (Varadarajan and David, 2003) than contrasting forms. Round-shaped and dark green colored fruits exhibit better resistance against mite attack than elongate-shaped fruits or nuts of other colors (Moore and Alexander, 1990).

Present study identifies few additional important traits related to mite resistance *i.e.,* color of the tepal and specific tepal weight, pinkish discoloration and resin production which are not reported by earlier workers. Role of these traits as a defense mechanism to protect coconut from mite infestation is envisaged in this study. It was found that red spectral values varried from 14 to 251, green spectral values from 12 to 237 and blue to vary from 5 to 183. Standard deviation was found to be low (18- 22) for blue spectral values (Table 4). There was considerable variation among individual plants within a variety. But negative correlation with mite damage value indicates the importance of the spectral value. However, there was no significant correlation between 3 and 5 months old fruit tepal with mite damage score. But spectral values especially Red max, Green max, 3*Red+Green max had significant negative correlation (>-0.4) with mite damage score values (Table 5). In the present study, it was found that the palms of MYD and COD had low level of damage due to mite. Red maximal spectral value was seen in MYD significantly (224) greater than other cultivars whereas the green fruited cultivars such GBGD, GDD (170) had lower score of red max. Green max spectra were maximum in COD, a pure orange fruited dwarf. Orange is a combination of red and green colors as per the colour wheel.

Table 4. Mean, range and standard deviation of maximal and minimal spectral values of red, green and blue in images of coconut fruit and tepal

	Red		Green		Blue	
	max	min	max	min	max	min
Mean	189	85	177	67	106	25
Max	251	207	237	183	183	92
Min	77	14	47	12	12	5
SD	34	42	33	39	22	18
CD (5%)	13	9	10	9	5	4

Color is an important trait in coconut palm but quantification of the same has been a neglected aspect. Coconut varieties display variation (green, orange, brown, yellow) in petiole, inflorescence, tepal and fruit. Petiole color is genetically controlled trait and used in identifying hybrid seedlings at nursery. Mite is a serious pest problem in coconut causing a crop loss of 25-30 per cent. Despite the importance of color, very little work is done on quantification of pigments involved and spectral details. Petiole color is by the action of two genes R, G in coconut (Bourdeix, 1999). Science of image analysis of color is explored in coconut up to sub pixel level of classification of mixed vegetation in a remote sensing imagery file (Palaniswami *et al*., 2006) and color of virgin coconut oil using infrared spectra (Yunus *et al*., 2009). Present study gives a method to quantify the color in coconut using the image analysis techniques. We also highlight the spectral maximal values of red and green as important associated traits to resistance to eriophyid mite based on the negative correlation (Table 5).

Table 5. Variation in selected spectral values and relationship to eriophyid mite damage

Cultivar	Red max	Green max	3R+ Gmax	Damage score
WCT	179	174	711	2.9
ADOT	196	187	775	1.3
COD	211	197	831	1.0
GDD	170	175	685	1.6
MYD	224	203	875	0.9
GBGD	203	199	808	1.5
LCOT	182	176	722	3.6
CD (5%)	13	10	48	0.5
Correlation	-0.47*	-0.43*	-0.47*	
t value	-2.12	-1.88	-2.10	

L* value of two month old fruits varied from 50.46 to 66.66 and of tepals 31.2 to 35.3. Penetrometer was employed to measure the thickness of the tepal and fruit whose readings varied from 14.6 to 28.9 in 3-month old tepals (Tables 6 & 7). Firmness values measured by penetrometer in 3 months old fruits and tepals of Benaulim variety (>38) and were higher than COD and MYD. Brightness of color of fruits and tepals was high as indicated by L* values of MYD. COD variety recorded high (red/green) (a* value of Hunterlab) >12 with potential to discriminate the variety from others (Tables 6 & 7).

Table 6. Color and firmness measured by Hunterlab and penetrometer observations in 2 months old coconut fruits and tepals

Variety	Tissue	L* (brightness)	a* (red/ green)	b* (yellow/ blue)	Pressure
Benaulim	Fruit	50.5	-2.2	40.5	32.9
MYD	Fruit	66.7	10.2	47.9	24.7
COD	Fruit	60.1	14.6	40.8	26.7
CD (5%)		4.1	5.0	2.8	2.7
Benaulim	Tepal	31.2	10.1	32.6	27.6
MYD	Tepal	35.3	7.6	27.9	16.4
COD	Tepal	31.4	12.1	30.3	24.9
CD (5%)		4.1	1.7	3.3	4.2

Table 7. Color and firmness measured by Hunterlab and penetrometer observations in 3 month old coconut fruits and tepals

Variety	Tissue	L* (brightness)	a*(red/ green)	b*(yellow/ blue)	Pressure
Benaulim	Fruit	52.1	-3.0	38.5	32.8
MYD	Fruit	71.4	8.6	48.9	25.4
COD	Fruit	64.7	16.6	42.1	25.9
CD (5%)		4.5	4.7	3.2	2.5
Benaulim	Tepal	32.8	9.0	38.8	28.9
MYD	Tepal	36.7	7.5	31.0	14.6
COD	Tepal	33.3	11.1	30.6	20.5
CD (5%)		2.1	1.4	2.9	4.3

Our studies explore the role of 21 traits and suggest the focus on seven traits related to tepals, resin cap space and color traits by multiple regression. Spectral values Red max, Green max, 3*Red + Green max had high significant negative correlation (>-0.4) with mite damage. Mite infestation is less in COD due to the high red/green value (a*) of fruit/tepal color. But the green fruited tress showed lower level of infestation possibly because of their firm tepal/fruit.

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