Correlation of volatile profiles of twenty mango cultivars with their susceptibilities to mango gall fly infestation

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Abstract

Mango gall fly (Procontarinia matteiana) is an orchard pest that parasitises flush leaves of mango and serious outbreaks may result in reduced fruit yield. The trigger for infestation is unknown, but terpenes emitted by the leaves appear to play a role in attraction. Metabolic profiles of three mango cultivars of varying susceptibility to mango gall fly attack were obtained by headspace profiling using GC-FID and GC-MS analysis. Chemometric models constructed from the data revealed that three terpenes, α- and β-pinene and camphene could be useful as biomarkers for susceptibility. Headspace profiles of twenty other cultivars, naturally exposed to gall fly, were obtained in the same way. Susceptibility or resistance of these cultivars was predicted using the developed orthogonal partial least squares model. Predictive outcomes were thereafter verified by visual examination of the leaves to detect gall formation, an indication of gall fly infestation. The model was found to predict the susceptibility or resistance of 90% of the cultivars accurately. This finding indicates the contributory role of the three terpene biomarkers in mango gall fly interaction and may direct future studies to determine their inter-relationship.

Keywords: Headspace profiles; Terpenes; Biomarkers; Metabolomics; Chemometrics; Mango gall fly; Susceptibility

1. Introduction

Mango (Mangifera indica) fruit is an important South African export crop, but optimum production is restricted by insect pests. The most prevalent are fruit fly, mango seed weevils, mango leaf webbers, citrus thrips and mango gall fly (Morton, 1987). Eleven mango gall fly (Procontarinia) species have been identified worldwide (Uechi et al., 2002), with Procontarinia matteiana representing the predominant species in South Africa. These insects pose a limited threat in regions where mangoes are indigenous, because parasites are able to control their numbers. However, in other areas where natural adversaries are not abundant, serious outbreaks of gall fly have been experienced (Sankaran, 1988). The extent of this threat is demonstrated by the rapid and uncontrollable spread of the disease once infected trees are introduced to a previously unaffected region. Procontarinia galls, for example, were first observed in Okinawa Japan in 2000, but infestation spread rapidly to six other islands and 78% of the mango growing areas in Okinawa were infested by 2002 (Uechi et al., 2002). In certain regions of South Africa including Hoedspruit and Nelspruit, extremely high incidences of gall fly infestation, are encountered, particularly in organic orchards. Heavily infested leaves have reduced photosynthetic capabilities, resulting in a lower fruit yield. Systemic pesticides can be applied to effectively control gall fly infestation but are expensive and not permitted in organic orchards.

The lifespan of the mango gall fly is but 1 day, during which the fly emerges from the gall, mates and lays eggs on flush leaves. These young leaves are favoured by the fly, since ovipositioning cannot easily take place on mature leaf surfaces, which are characterised by thick, protective epidermal wax and cutin layers. Larvae hatching from the eggs then tunnel into the young leaves, resulting in the formation of galls that house the developing larvae. Once the mature gall fly has fully formed within the wart-like structure, a signal to emerge prompts the insect to exit. Emergence of the adult fly from the gall and the

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presence of young flush occur concurrently. Volatile compounds, produced by the flush leaves, possibly alert the insects to the availability of flush leaves for ovipositioning. It is well documented that volatile organic metabolites of plants play an important role in their communication and defense (Assad et al., 1997; Wool, 2005).

Mango gall fly was not of concern to mango producers in the past, since only flush leaves are attacked and the fruit is left unharmed (Sankaran, 1988). However, a new species, Procontarinia frugivora that attacks only fruit, was identified in 2004. Although this species currently appears to be localised in the Luzon Island of the Philippines (Gagné and Medina, 2004), there are concerns that the pest may spread to other mango-producing areas. The identification of this species has placed the mango gall fly in the spotlight as a potential threat to mango production worldwide.

Some mango cultivars are more resistant to gall fly infestation than others (Schoeman et al., 1996). In this study, a chemometric model to predict the susceptibility of mango cultivars to gall fly infestation was developed using the gas chromatographic (GC) profiles of the volatile compounds emitted by three cultivars displaying varying degrees of susceptibility. The cultivar 'Heidi' typically displays severe gall formation, 'Keitt', is susceptible, but only develops pseudogalls (incomplete development of galls), while 'Sensation' is resistant to gall fly attack. The aim was to use these three cultivars to identify terpene biomarkers involved in gall fly attraction that could account for the susceptibility or resistance of the cultivars. In addition, headspace GC profiles of twenty other mango cultivars were obtained and the susceptibilities of these cultivars were predicted using an orthogonal partial least squares (O-PLS) model. The predictive outcomes were verified by visual examination of the leaves to detect gall formation.

2. Materials and methods

2.1. Sampling of plant material

Mature and flush leaf samples, two sets of five samples of each of the three selected cultivars ('Heidi', 'Keitt' and "Sensation') were obtained monthly throughout two full growing seasons (August 2006 to August 2008) from trees in the Bavaria and Moriah Fruit Estate orchards in Hoedspruit, Mpumulanga. Flush leaves are not available throughout the season and a forced flush was obtained by pruning the trees a week before sampling. All samples were collected in the mornings when temperatures ranged from 23 to 28 °C. The headspace of each sample was obtained in situ as detailed by Augustyn et al. (2010). Briefly, the headspace volatiles, accumulated for 1 h in an aluminium foil bag placed over the leaves, were drawn over 197 mg Tenax TA® adsorbent (Markes International Ltd., Pontyclun, UK) in a stainless steel tube.

Additional twenty cultivars, growing in an organic orchard and naturally exposed to gall fly, were sampled in the same way. Headspace samples from mature leaves of the cultivars 'Heidi', 'Keitt' and 'Sensation' were also collected from the same orchard. Due to orchard constraints, no forced flush was available from this locality.

2.2. Chromatographic analysis

Headspace samples were desorbed with a thermal desorption unit (Unity, Markes International Ltd., Pontyclun, UK) as described by Augustyn et al. (2010) and the volatilised sample was transferred to a Varian 3800 gas chromatograph (Walnut Creek, CA, USA). Terpenes were separated on a Phenomenex Zebron ZB-5 column (cross-linked 5% phenyl polymethylsiloxane) 30 m × 0.25 mm i.d. × 0.25 μm film thickness, using the instrumental conditions described previously (Augustyn et al., 2010).

The analyses were repeated on replicate tubes using the same chromatographic conditions with an Agilent gas chromatograph (model 6890 N, Chemetrix, SA) coupled to a model 5975B mass selective-detector (Augustyn et al., 2010). Compounds were identified with the aid of a NIST version 5 library and the identities were verified by analysis of authentic reference standards (Sigma-Aldrich, UK).

2.3. Chemometric analysis

The percentage peak areas of the terpenes, relative to that of Δ^3-carene, were obtained from both gas chromatography-flame ionisation detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) results and used for chemometric analysis. Multivariate analysis of GC-FID and GC-MS data was done by constructing various chemometric models to determine the relationships between the datasets. SIMCA-P + (12.0) software (Umetrics, Umeå, Sweden) was used to perform chemometric computations after applying univariate scaling to the data. Scatter and loading plots, as well as S- and SUS-plots were set up to visualise results and predict possible terpene biomarkers. Analysis by orthogonal partial least squares (O-PLS) was done to establish a model, using the levels of identified biomarkers in both mature and flush leaves to enable the prediction of susceptibility or resistance of mango cultivars to gall fly attack. The model was internally cross validated using seven cross validation groups and evaluating the obtained R^2 and Q^2 values. External validation was done by removing ten susceptible and ten resistant data sets and using these to test the predictive ability of a new model constructed from the remaining data.

3. Results and discussion

Volatile profiles of the two leaf types, flush and mature, of the three selected cultivars were obtained over two full seasons to determine if volatile emissions play a role in gall fly attraction. The most prominent peak on the chromatograms obtained for all three cultivars was that of Δ^3-carene, representing between 60 and 70% of the total percentage peak areas. All terpene values were subsequently calculated relative to that of Δ^3-carene, and expressed as a percentage peak area. Headspace profiles demonstrated that flush leaves emitted higher concentrations and a larger variety of terpene components than mature leaves. To demonstrate the marked difference in the emissions of mature and flush leaves, the peak area ratios of Δ^3-carene to the mass of oven dried 'Heidi' flush and mature leaves, were compared. Flush leaves emitted 3.8 μg of Δ^3-carene per g of dried leaves compared to a mere 0.06 μg of Δ^3-carene per g of dried mature leaves of the same tree, sampled at the same time. 'Heidi', the susceptible and 'Keitt', the pseudogall-forming cultivar displayed similar volatile profiles, while that of the resistant cultivar 'Sensation' was completely different (Table 1). The foremost differences between susceptible and resistant cultivars were the levels of α- and β-pinene and camphene. These terpenes were either absent or present at very low levels, in the resistant cultivar. The levels of α- and β-pinene increased as the season progressed, reaching maximum concentrations in February, coinciding with the major flush of mango trees in South Africa. Other researchers reported similar observations for other species (Hall and Langenheim, 1986; Harborne, 2001). Headspace profiles of holly (Ilex opaca) leaves at different stages of maturity were found to differ (Harborne, 2001). Hall and Langenheim (1986) reported that the highest concentrations of terpenes (mg/g dry weight) were emitted by Sequoia sempervirens in the first six months following leaf flush, but that the concentrations gradually declined as the leaves reached maturity.

In this study, qualitative and quantitative data for as many compounds in the headspace samples as possible were obtained and statistically processed using principal component analysis (PCA) and orthogonal partial least squares (O-PLS) analysis; an approach frequently used in chemometric analysis (Eriksson et al., 2006; Wishart, 2008). These models are used to distinguish samples and enable the identification of those variables that contribute mainly to differences observed (Eriksson et al., 2006). Such models, constructed from the GC-FID and GC-MS data of 'Heidi' and 'Keitt', indicated clustering according to leaf type (scatter plots not shown). These results suggest that there are inherent chemical compositional differences between flush and mature leaves. A PCA model could not be constructed for the resistant cultivar, 'Sensation', because the data did not reveal clustering or variation between flush and mature leaves.

Partial least squares (PLS) is a regression technique that enhances the separation between groups of observations by rotating PCA components to obtain maximum separation between classes. In PLS, a correlation between the independent observations (X) and the dependent data (Y) is obtained (Eriksson et al., 2006). A modification of the PLS method, orthogonal-PLS (O-PLS), can be used to point out those variables that are responsible for class discrimination. An O-PLS model for 'Heidi' (susceptible) and 'Sensation' (resistant) was constructed using normalized GC-FID and GC-MS data. The scatter plot (Fig. 1A) indicates that a good separation based on class (Y-variable), selected as either 'resistant' or 'susceptible', was obtained ($R^2Y = 0.866$). The high Q^2 value (0.830) confirms the high predictive ability of the model while the lower R^2X value (0.542) indicates some separation between the X-variables. However, the separation of the X-variables (terpenes) was not as pronounced as that of the Y-variables (classes), since the resistant and susceptible cultivars are clearly distinguished in the first component. This result can be attributed to the differences in the headspace volatiles emitted by the leaves. A second O-PLS model, constructed from the GC-FID and GC-MS data obtained from 'Keitt' and 'Sensation', allowed mid-susceptible and resistant cultivars to be compared. Once again, clustering according to class was clearly indicated (Fig. 1B) through separation in the first component ($R^2X = 0.331$; $R^2Y = 0.746$, $Q^2 = 0.703$). In contrast, no valid model ($R^2X = 0.316$; $R^2Y = 0.209$, $Q^2 = 0.184$) was obtained to describe separation between susceptible and mid-susceptible classes. This result was expected, as both susceptible and mid-susceptible cultivars attract gall fly and display similar volatile profiles. The difference in susceptibility of 'Heidi' and 'Keitt' to gall fly infestation is therefore unrelated to the differences in insect attraction by emitted terpenes. We believe that in the case of 'Keitt,' development of the insect inside the gall is deterred, perhaps as a result of the presence of non-volatile secondary metabolites present in the leaves. Data derived from these three models indicate that the observed separation between

Table 1
Average percentage peak areas (n = 5) relative to Δ^3-carene, of the major components identified by GC-FID in headspace samples of mature and flush leaves of the three cultivars collected in February 2007. Reference component is indicated in bold-face.

Terpene	'Heidi' (susceptible)		'Keitt' (pseudogall)		'Sensation'(resistant)	
	Mature	Flush	Mature	Flush	Mature	Flush
α-Pinene	18.8	20.1	14.9	16.4	1.1	1.3
Camphene	2.1	1.1	2.0	1.4	0.0	0.0
β-Pinene	6.6	6.8	5.4	5.2	0.0	0.0
Myrcene	6.9	8.0	3.5	6.0	2.6	4.9
Phellandrene	0.43	0.4	1.4	0.9	1.3	1.1
Δ^3-Carene	100	100	100	100	100	100
Limonene	6.0	7.8	4.3	4.2	2.7	4.5
Ocimene	0.47	0.7	0.85	0.0	0.0	0.1
Terpinolene	3.4	3.9	1.6	2.6	2.4	5.0
Caryophyllene	0.62	0.4	4.9	0.0	0.4	0.1
α-humulene	1.7	0.1	0.15	0.0	0.3	0.1
γ-Gurjunene	0.68	0.1	5.3	0.0	0.3	0.0

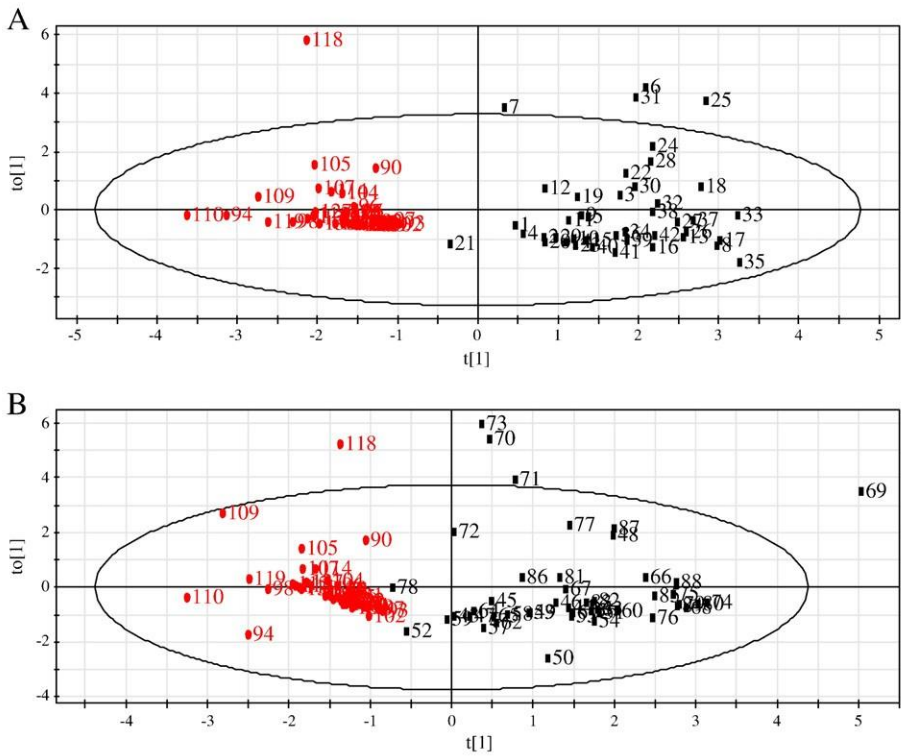

Fig. 1. O-PLS score plots of the first two principal components constructed from GC-FID and GC-MS data of 'Heidi' and 'Sensation'. 1A: O-PLS score plot comparing the susceptible cultivar 'Heidi' (black) to the resistant cultivar 'Sensation' (red) (R^2X for the first component = 0.331 and R^2X for the orthogonal component = 0.211). 1B: O-PLS score plot comparing the mid-susceptible cultivar 'Keitt' (black) to the resistant cultivar 'Sensation' (red) (R^2X for the first component = 0.283 and R^2X for the orthogonal component = 0.277).

classes (resistant or susceptible) is related primarily to inter-cultivar differences. The R^2X values indicate that there is some variability in the terpene levels within a cultivar.

S-plots derived from O-PLS are useful for the identification of biomarkers and are often used in metabolomics (Wishart, 2008). From the S-plot of susceptibility vs resistance, α-and β-pinene and camphene were identified as biomarkers (Fig. 2A). The position of these terpenes on the S-plot, upper right hand, indicates correlation with susceptibility. The x-axis describes the degree of influence of each terpene, while the y-axis expresses the reliability of each terpene as a contributing factor in the model. Positive values have a high reliability, implying high effect and low uncertainty. The S-plot indicates that the identification of α-and β-pinene and camphene as biomarkers can be made with confidence. In contrast, values of low magnitude are at a higher risk as biomarkers. Phellandrene, for example, could not be labeled a biomarker since a low magnitude and a low reliability were assigned to the terpene in the model. Biomarkers identified by the S-plot are statistically significant and should be further investigated (Eriksson et al., 2006). The terpenes identified as biomarkers show up as prominent peaks in the GC chromatograms of the headspace profiles of the two susceptible cultivars and clearly represent a considerable percentage of their emissions. Biomarkers have previously been implicated in plant–insect interaction. The terpenes, α-pinene, terpinolene and (+)-3-carene, were found to be involved in the attraction of the pine shoot beetle, Tomicus piniperda (L), an exotic pest of pine, Pinus spp. (Poland et al., 2003).

The finding that α- and β-pinene as well as camphene can be considered biomarkers for susceptibility was strengthened by the loading column plot, with jack-knifed confidence intervals (Fig. 2B). These confidence intervals reflect the uncertainty associated with the prediction and are directly correlated with reliability. The three terpenes identified as biomarkers all presented small confidence intervals. Phellandrene displayed a negative loading and is therefore negatively correlated with susceptibility. The confidence interval indicated for this terpene (Simca defaults α to 0.05) crosses the zero mark, thereby reflecting a large degree of uncertainty. Metabolites with confidence limits that do not cross zero are 95% statistically safe (Eriksson et al., 2006). When comparing 'Heidi' to 'Keitt' (susceptible to mid-susceptible), α-pinene, β-pinene and camphene were again identified as biomarkers, while two additional terpenes, α-humulene and gurjunene, were distinguished as possible biomarkers (Fig. 2C).

SUS-plots are used to compare two models to determine if the same biomarkers are relevant to both models. Comparing the models for susceptibility vs resistance, and for mid-susceptibility vs resistance, α-and β-pinene and camphene were once again strongly correlated with susceptibility (Fig. 2D).

A previous study made use of metabolic profiling to reveal resistance-related metabolites to Fusarium head blight on

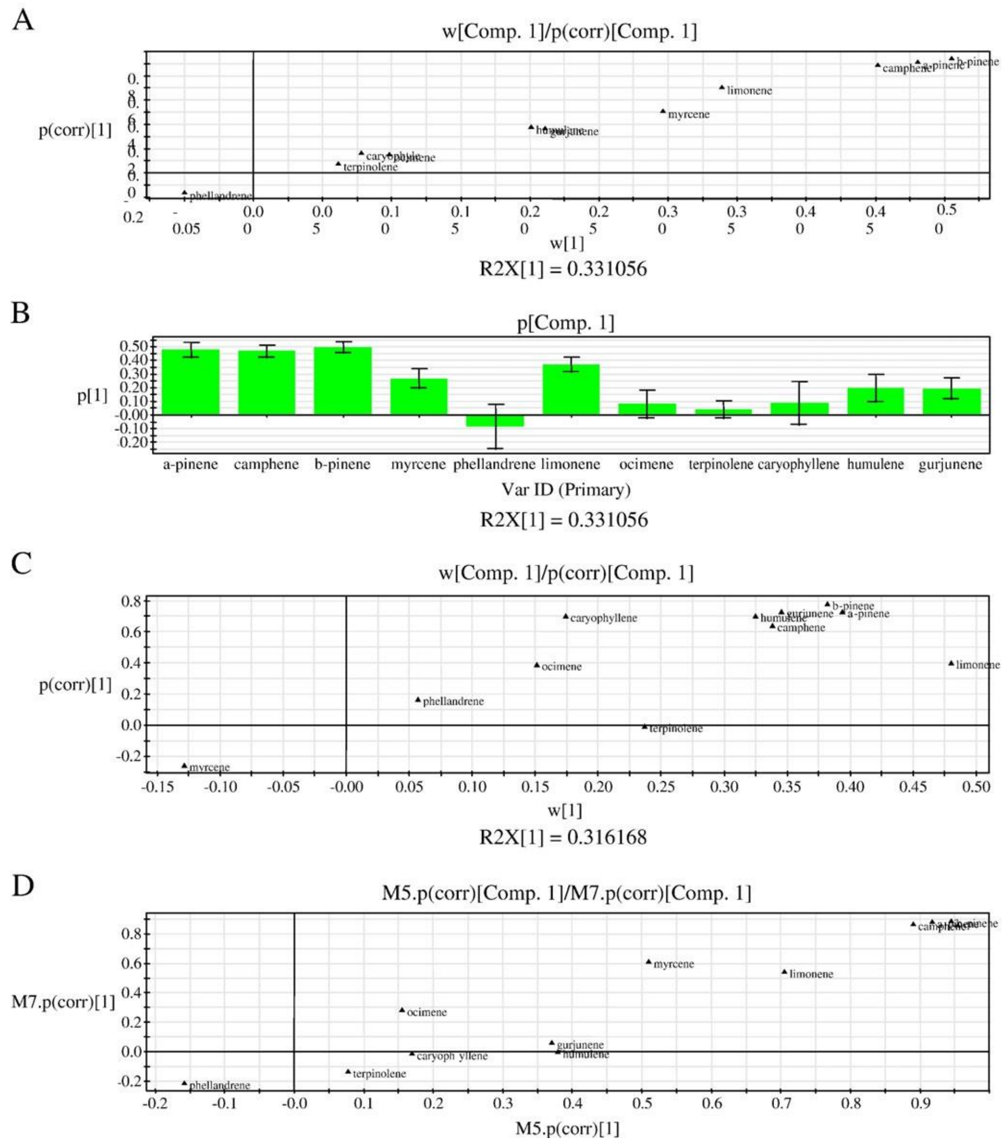

Fig. 2. S-plots, loading bi-plot and SUS-plot to determine biomarkers for susceptibility to gall fly. 2A: Biomarker identification using the O-PLS S-plot comparing susceptibility to resistance with $R^2X = 0.331$. 2B: Loading column plot with confidence limits for each of the terpenes present in the headspace of susceptible and resistant cultivars. 2C: Biomarker identification using S-plots comparing the susceptibility to mid-susceptible (pseudogall) with $R^2X = 0.316$. 2D: SUS-plot comparing the susceptible/resistant model to the mid-susceptible/resistant model.

wheat, allowed screening of new cultivars before cultivation of the wheat line (Hamzehzarghani et al., 2008). Their findings correspond with the results of this study whereby biomarkers associated with susceptibility to gall infestations were identified with the aid of chemometric models. These biomarkers enabled the development of a prediction model that was applied to predict the susceptibility of other cultivars. The prediction model was based exclusively on the chromatography data of the biomarkers (α- and β-pinene, camphene) identified. The model exhibited an excellent fit with regard to class ($R^2Y = 0.989$) and variation in the terpene levels ($R^2X = 0.805$), and displayed a high reliability ($Q^2 = 0.777$). Internal cross validation ($R^2X = 0.897$) confirmed the validity of the model. It was also externally validated by removing 20 data sets from the original data. Following construction of a new model from the remaining data, this model was challenged with the test set. The miscalculation table indicated that 100% of the susceptible samples and 90% of the resistant samples were correctly predicted. The value (6×10^{-5}) for the Fisher's exact probability reflects the very small probability of obtaining such a classification by chance (Eriksson et al., 2006). It was therefore concluded that this model was appropriate for prediction of susceptibility of mango cultivars, based on the levels of the three terpenes emitted by their leaves. Both, mature and flush leaves of 'Heidi' (susceptible) and 'Sensation' (resistant) were used to construct the prediction model. Mature leaves of 'Heidi', 'Keitt' and 'Sensation' sampled from an organic orchard yielded accurate predictions concerning their susceptibilities to gall fly. This indicates that the model is suitable for predictions based on mature leaf profiles. Headspace profiles of mature leaves of 20 other cultivars, sampled on the same day, were obtained and tested against the model. The selected trees from an organic orchard were all exposed to the same conditions and prevalence of gall fly.

The predicted score column plot indicates cultivars that are positively correlated and those that are negatively correlated with susceptibility (Fig. 3A). From the plot it can be seen that ʹAlphonseʹ, ʹJulieʹ, ʹKensingtonʹ, ʹSuzzieʹ, ʹSunshineʹ, ʹNam-docmaiʹ, ʹManillaʹ and ʹPinsaimunʹ are negatively correlated with susceptibility and were therefore predicted to be resistant. On the other hand, the model classified ʹAfrican Goldʹ, ʹAmeeriʹ, ʹFairchildʹ, ʹHoney Goldʹ, ʹIrisʹ, ʹIrwinʹ, ʹJoaʹ, ʹKentʹ, ʹMayaʹ, ʹSugarʹ, ʹTommy Atkinsʹ and ʹZillʹ as susceptible cultivars. The DModX plot (Fig. 3B) represents the fit of the predicted values to the model. The cultivars ʹIrisʹ, ʹJoaʹ, ʹMayaʹ, ʹSugarʹ and ʹTommy Atkinsʹ displayed values exceeding Dcrit = 2.275. This poor fit to model obtained can be explained by the very high values of α- and β-pinene emitted by these cultivars (Table 2) compared to the generally lower values (20.44 ± 8.19%) emitted by ʹHeidiʹ and ʹKeittʹ, the two cultivars used to develop the model. ʹIrisʹ and ʹMayaʹ displayed α-pinene, rather than Δ^3-carene, as the major peak, while ʹTommy Atkinsʹ and ʹSugarʹ emitted α-pinene with corresponding values of 87% and 84%, respectively. The very high values of α-pinene do not fit the model well, thus explaining the observed distance between the model and the predicted value for the cultivar. Predictions are still possible for observations that fall outside the model tolerance volume in X-space (Eriksson et al., 2006). However, the extrapolation of the model outside its range of validity renders these predictions less precise than predictions for observations that fit the model. Alpha-pinene can be considered a good biomarker, because all cultivars displaying high levels of the terpene were classified as highly susceptible to gall fly attack by the model. Although ʹJoaʹ emitted α-pinene at levels within the range of values used to set up the model, the poor fit to model of this cultivar can be explained by the β-pinene values (11%), which were much higher than those applied in the model (5.66 ± 1.81%). The predictive ability of the model for β-pinene was thus confirmed.

To verify the prediction of susceptibility or resistance of the various cultivars to gall fly attack, visual inspection of the leaf surfaces for the presence of galls was done. The occurrence of galls, an indication of earlier insect activity, was graded. Very high incidences were allocated a value of 2, while pseudogalls were designated a grading of 1 and the absence of galls was indicated by 0 (Table 2). The predictive outcomes were correlated with susceptibility, confirmed by the presence of galls. Higher levels of the biomarkers, particularly α- and β-pinene, correlated with a high incidence of galls. Some cultivars, with α- and β-pinene levels similar to those of the susceptible varieties presented with pseudogalls, indicating gall fly infestation, but subsequent inability of the larvae to develop. In contrast, ʹKensington Prideʹ exhibiting similar levels of α- and β-pinene, was expected to be highly susceptible, but this cultivar was not classified as either resistant or susceptible by the model (Fig. 3A). Visual inspection of the leaves did indicate minor gall fly activity, albeit significantly less than that of susceptible cultivars. The high levels of terpinolene detected in the headspace offers a possible explanation for this finding. Australian cultivars, derived from ʹKensington Prideʹ, are

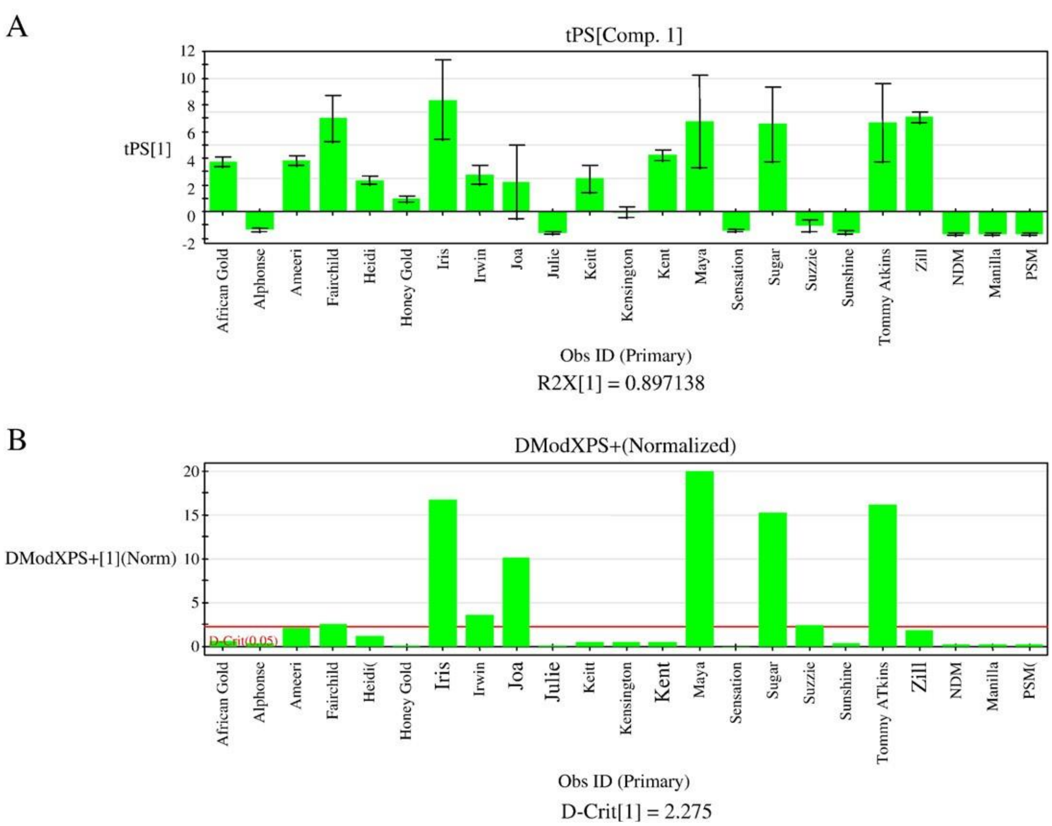

Fig. 3. Predicted score plot and DModX plot for the prediction of susceptibility to gall fly infestation of mature leaves of the three selected cultivars and 20 other cultivars. 3A: Predicted score plot with $R^2X = 0.897$, 3B: DModX plot with Dcrit = 2.275.

Table 2
Comparison between biomarkers, α- and β-pinene and camphene percentages, relative to the major component indicated in the table, and gall fly susceptibility of 23 mango cultivars.

Cultivar	α-Pinene (%)	Camphene	β-Pinene (%)	Gall fly susceptibility	Major component
Iris	100	2.9	14	2	α-Pinene
Maya	100	1.9	9.6	2	α-Pinene
Fairchild	100	7.8	12	2	α-Pinene
Sugar	84	1.9	10.3	2	Δ^3-Carene
Tommy Atkins	86.5	1.7	10	2	Δ^3-Carene
Joa	17	0.6	11	2	Δ^3-Carene
Zill	56	5.3	16	2	Δ^3-Carene
African Gold	35	4	10	2	myrcene
Heidi	34	3.1	7.7	2	Δ^3-Carene
Sunshine	2.3	0	0	2	Δ^3-Carene
Honey Gold	20	1.9	4.8	2	Δ^4-Carene
Ameeri	41	3.0	9.4	2	Δ^3-Carene
Kent (Pseudo)	37	3.1	10	1	Δ^3-Carene
Irwin (Pseudo)	19	3.1	9.0	1	Δ^3-Carene
Keitt (Pseudo)	16.7	1.5	4.3	1	Δ^3-Carene
Kensington Pride	16	1.5	3.0	1	terpinolene
Suzzie	10	0.0	0.4	1	Δ^3-Carene
Alphonse	4.0	0.2	0.4	0	Δ^3-Carene
Julie	1.4	0	0.0	0	Δ^3-Carene
Sensation	1.3	0.0	0.0	0	Δ^3-Carene
Namdoc mai	0.0	0.0	0.0	0	
Pinsaimun	0.0	0.0	0.0	0	
Manilla	0.0	0.0	0.0	0	

devoid of gall fly and all exhibit terpinolene as a major compound in the headspace profiles. This terpene could possibly act as a deterrent to infestation (Fig. 3A).

The other cultivar that the model was unable to predict correctly was 'Sunshine'. According to the model, 'Sunshine' was classified as resistant, and the levels of biomarkers confirmed this prediction. However, 'Sunshine' displayed severe gall formation, indicating that other factors may be involved. The variety emitted higher levels of α-humulene (11%), compared to 'Heidi' and 'Keitt' (values ranging from 0 to 9.0%) suggesting that this terpene may contribute to the incorrect classification of the cultivar. Humulene showed up as a potential biomarker on the S-plot comparing susceptible and mid-susceptible cultivars (Fig. 2C), but data regarding humulene was not included in the prediction model leading to a poor result for 'Sunshine'. To improve the prediction model, additional cultivars with more variable levels of terpenes should be sampled and added to the prediction set.

Finally, 'Namdocmai', 'Manilla' and 'Pinsaimun' displayed no prominent volatiles and only low levels of terpenes were emitted by the leaves of these cultivars. This lack of terpene biomarkers corresponds to resistance in these cultivars, which was confirmed by the absence of galls on the leaves.

4. Conclusion

This investigation has proved that certain terpenes emitted by mango flush are associated with the susceptibilities of the cultivars to gall fly infestation. A chemometric model using GC data was used to identify biomarkers, α-pinene, β-pinene and camphene, associated with susceptibility. An O-PLS model developed using the biomarker data of three selected cultivars was found to predict the susceptibility or resistance of 20 other cultivars with a 90% accuracy. In the future, this model can be used to predict the susceptibility of new mango cultivars intended for cultivation in areas where gall fly is of concern.

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