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# 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 $\Delta^3$-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 $\Delta^3$-carene, representing between 60 and 70% of the total percentage peak areas. All terpene values were subsequently calculated relative to that of $\Delta^3$-carene, and expressed as a percentage peak area. Headspace profiles demonstrated that flush leaves emitted