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Research article

Chemical composition and water permeability of the cuticular wax barrier in rose leaf and petal: A comparative investigation

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ABSTRACT

Cuticular wax is the main transpiration barrier against uncontrolled water loss for all aerial plant organs. This study presents water permeability and chemical composition of the cuticle on the petals and leaves of two cultivars of *Rosa chinensis* ('Movie star' and 'Tineke'). Numerous cultivar- and organ-specific differences, such as the water permeability and total cuticular wax, were detected among rose petals and leaves. Overall, the permeability to water is higher in petals than in leaves, varying between 1.8×10^{-5} m s^{-1} ('Tineke' leaves) and 1.0×10^{-4} m s^{-1} ('Tineke' petals). The cuticular wax coverage ranges from 4.9 μg cm^{-2} ('Tineke' petals) to 13.2 μg cm^{-2} ('Movie star' petals). The most prominent components of the waxes are *n*-alkanes with the odd-numbered chain lengths C_{27} and C_{29} in petals, and C_{31} and C_{33} in leaves. The lower water permeability of leaves is deduced to be associated with the higher weighted average chain length of their acyclic cuticular waxes. This study on transpiration via the cuticular wax barrier of the leaf and petal of rose provides further insight to link the chemical composition to the cuticular transpiration barrier properties.

1. Introduction

The plant cuticle is a thin lipophilic film sealing the outer epidermal surfaces of most leaves, flowers, fruits and non-woody stems. The primary physiological function of the plant cuticle is to prevent uncontrolled water loss as a result of biotic and abiotic stress (Riederer and Schreiber, 2001). The cuticle also plays an important role in regulating gas exchange, chemical transportation, protection against invasion by microorganisms, and shielding of detrimental ultraviolet radiation (Müller and Riederer, 2005). As the plant cuticle is a chemical and physical structure, its functional properties are largely determined by its chemical composition and structural arrangement. The plant cuticle is mainly composed of cutin polymers and waxes. The cutin polymers are polyesters that are esterified from monomers consisting of C_{16} and C_{18} fatty acids, and hydroxy fatty acids with additional mid-chain secondary groups such as hydroxy, carboxy, epoxy, and oxo groups (Fich et al., 2016). The cuticular waxes are embedded within the cutin polymers (intracuticular waxes) and deposited on its outer surface (epicuticular waxes). They have been found to be dominated by straight-chain aliphatic compounds such as fatty acids, primary alcohols, *n*-alkanes, aldehydes, and alkyl esters; and cyclic compounds such as triterpenoids and sterols (Samuels et al., 2008).

Depending on the plant species or cultivar, and on the organ, the total amount and composition of the cutin monomers and waxes vary widely (Martin and Rose, 2014). These differences account for the differences in barrier properties, especially regarding the transpiration barrier function of the cuticle. It has long been known that cuticular waxes, rather than cutin polymers, form the crucial barrier against uncontrolled water loss (Schönherr, 1976; Riederer, 1995). Structurally, the barrier properties of the wax layer have been proposed to be the result of its arrangement into partially highly ordered crystalline and amorphous zones (Reynhardt and Riederer, 1994). This model indicates that the tight arrangement of aliphatic components predominantly contributes to the layer's high impermeability to water and organic and inorganic substances (Schreiber and Schonherr, 2009). Due to the compositional diversity of cuticular waxes, the properties of the transpiration barrier differ widely among different plants and organs (Schreiber and Riederer, 1996; Jetter and Riederer, 2016).

The flower of many plants is thought to help attract insects and assist pollinators. It has been proved that petals are covered by cuticles, which are highly comparable to those in other organs (Whitney et al., 2011). Notably, several studies have reported cuticular wax

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compositions for different petal cuticles. For example, *Solanum tuberosum* L. (potato) and *Antirrhinum majus* L. (snapdragon) have been shown to contain relatively high concentrations of *n*-alkanes and methyl-branched alkanes (Goodwin et al., 2003; Guo and Jetter, 2017). Similarly, the major cuticular wax components of *Vicia faba* L. (faba bean) and *Rubus idaeus* L. (raspberry) petals were *n*-alkanes (Griffiths et al. 1999, 2000). Primary alcohols (21%) and *n*-alkanes (29%) were found in similar amounts in the wax layer of *Taraxacum officinale* (L.) Weber ex F.H.Wigg. (dandelion, Guo and Jetter, 2017). In the petal wax mixture of *Solanum tuberosum* L. (potato), the relatively abundant components were *n*-alkanes (35%), 2-methylalkanes (43%), and 3-methylalkanes (19%) (Guo and Jetter, 2017).

During the past half-century, the barrier properties of the cuticular wax layers of leaves have been elucidated in much detail. In contrast, few studies have addressed the barrier properties of petal cuticles for transpiration taking into account their chemical composition. To date, only one study on *Cosmos bipinnatus* Cav. petals (Buschhaus et al., 2015), has attempted to correlate the surface water barrier properties with the wax components. The wax layer of *C. bipinnatus* petals has relatively high concentrations of C_{22} and C_{24} fatty acids and primary alcohols, which are much shorter than those in leaves. Accordingly, the petal cuticle exhibited weaker transpiration barrier properties than leaf cuticles. In addition, a comparative study on the chemical composition of cuticular waxes between two or more organs such as leaf, stem, flower, and fruit has been conducted (Guo and Jetter, 2017). However, the contribution of the cuticular components to cuticle functions, particularly the transpiration barrier properties, has not been studied comprehensively for petals. To help fill the gap in our understanding of surface water barrier properties and wax compositional diversity in petal cuticles, the present study aims to comparatively investigate water permeability and cuticular waxes of the leaf and petal of two rose cultivars of *Rosa chinensis*, 'Movie star' and 'Tineke'.

2. Materials and methods

2.1. Plant materials

Petals of two rose cultivars (*R. chinensis* Jacq. 'Tineke' and *R. chinensis* 'Movie star') were selected as the experimental materials. Rose seedlings of these two cultivars were grown in pots with soil under a 12-h day/12-h night light cycle at 20 ± 2 °C and 60 ± 2% relative humidity in a climate chamber at Zhongkai University of Agriculture and Engineering. The plants were watered daily to keep the soil moist. Leaves and petals were picked three to seven days after flowering started for analysis of the water permeability.

2.2. Determination of water permeability

The water transpiration of whole petals was determined to measure water loss over time. Ten fresh petals without any defects from each species or cultivar were selected. Before measurement, the petiole of the petals was sealed with paraffin wax (melting point 60 °C). Samples were kept at room temperature. The temperature and relative humidity of the surrounding atmosphere were monitored using a digital thermometer (Anymetre, Guangzhou, China). The weight loss was recorded every 30 min for 4–6 h using a digital balance with a precision of 0.1 mg (BSA-224S, Sartorius, Beijing, China).

The transpiration rate (flux of water vapour; F in g $m^{-2} s^{-1}$) was obtained from the change in the fresh weight of the samples (W in g) over time (Δt in s) and surface area (A in m^2).

$$F = \frac{\Delta W}{\Delta t \times A}$$

The surface area of the petal (A) was obtained by scanning. The water permeance (P in m s^{-1}) was calculated from the transpiration rate (F) divided by the driving force:

$$P = \frac{F}{C^*_{wv}(a_{petal} - a_{air})}$$

The water vapour saturation concentration at the actual petal temperature (water vapour content of air at saturation; c^*_{wv}) was obtained from tabulated values (Nobel, 2009). The water activity for air (a_{air}) was the relative humidity. The water activity in petal (a_{petal}) was assumed to be unity (Burghardt and Riederer, 2003).

2.3. Cuticular wax extraction

To extract the cuticular waxes from the surface of leaves and petals, whole petals and leaves were dipped in chloroform. To avoid contact of the solvent with the pedicel, the petal and leaf were dipped into chloroform vertically for 30 s. Each sample was extracted three times consecutively, and the three extracts were combined. *n*-Tetracosane was added to the extracts as an internal standard. The solvent was evaporated under a gentle stream of nitrogen.

2.4. Wax composition analyses

Prior to further analyses, the dried samples were derivatised with *N, O*-bis (trimethylsilyl) trifluoroacetamide in pyridine at 70 °C for 30 min. To determine the quantity of wax components, samples were analysed using a capillary gas chromatograph (7820A, GC System; Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column (30 m × 0.32 mm, DB-1ms, 0.1 μm film; J&W Scientific, Agilent Technologies). The GC oven was held at 50 °C for 2 min, raised by 40 °C min^{-1} to 200 °C, held at 200 °C for 2 min, and then raised by 3 °C min^{-1} to 320 °C and held at 320 °C for 30 min. The carrier gas was hydrogen. The area of the peaks was compared with that of the internal standard to achieve the quantity of wax components.

The wax components were analysed using a temperature-controlled capillary gas chromatograph equipped with a mass spectrometric detector (m/z 50–750, MSD 5975; Agilent Technologies) under the same gas chromatographic conditions but with helium as carrier gas. Single compounds were identified based on their electron ionization mass spectra using authentic standards, the Wiley 10th/NIST 2014 mass spectral library (W10N14; John Wiley & Sons) or by interpretation of the spectra according to their retention times and/or by comparison with literature data.

2.5. Statistical analysis

Statistical analyses were performed using SPSS (23, IBM Corp., Armonk, NY, USA) and SigmaPlot 10 (Systat Software, Inc., San Jose, CA, USA). The normal distribution of data was tested via a Shapiro – Wilk or Kolmogorov–Smirnov normality test (significance level, $p < 0.05$). Comparison analyses were performed by one-way analysis of variance (ANOVA). SigmaPlot 10 was used to depict the graphs.

3. Results

3.1. Water permeances and resistances

The water permeability for the petals and leaves of the two cultivars 'Tineke' and 'Movie star' of *R. chinensis*, was determined. Owing to the occurrence of stomata on the abaxial surface of both petals and leaves, cuticular transpiration was indicated by minimum conductance (Burghardt and Riederer, 2003). The initial conductance was very high, and after reaching a certain relative water deficit (RWD), the change of conductance was constant and linear (Supporting information Fig. S1). In general, the minimum conductance for rose petals was significantly higher than in leaves. The overall minimum conductance of petals was 4.1×10^{-5} m s^{-1} ('Movie star') and 1.0×10^{-4} m s^{-1} ('Tineke'),

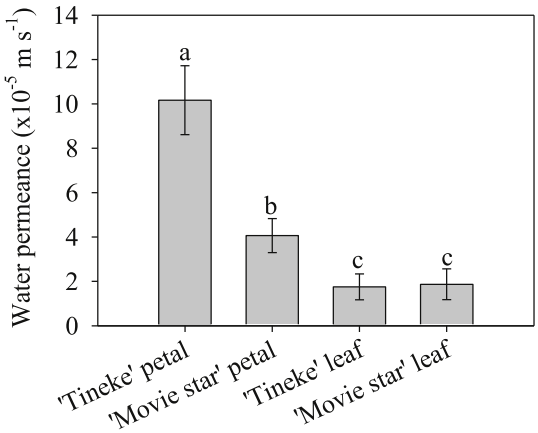

Fig. 1. Permeance for water via the surface of petal and leaf of *Rosa chinensis* 'Movie star' and 'Tineke'. Data are given as means ± standard deviation ($n = 10$). The different lowercase letters on the bars indicate significant differences ($p < 0.05$).

whereas a lower minimum conductance was detected for leaves, of 1.9×10^{-5} m s^{-1} ('Movie star') and 1.8×10^{-5} m s^{-1} ('Tineke'), respectively (Fig. 1).

In addition, to characterize the contribution of the cuticular layer to the overall barrier properties, the corresponding overall water resistance was calculated for the petals and leaves. The water resistance of the petals was higher than that of leaves in 'Tineke' and 'Movie star' (Fig. S2). These results indicate that the overall cuticular barrier property for water transpiration of rose leaves is better than that of petals.

3.2. Wax coverage and composition

The chemical composition of the cuticular wax was analysed in both leaves and petals for comparison. Overall, the cuticular wax layer coating the petal was 13.2 μg cm^{-2} for 'Movie star', and 4.9 μg cm^{-2} for 'Tineke', a significantly lower wax coverage by comparison (Fig. 2). The petal waxes mainly consisted of typical very-long-chain fatty acids and their derivatives (VLCFAs), including fatty acids, primary alcohols, aldehydes, *n*-alkanes, and branched *n*-alkanes, *n*-alkenes, etc. (Fig. 3 A B, Supporting information Fig. S3 and Table S1). In particular, *n*-alkanes

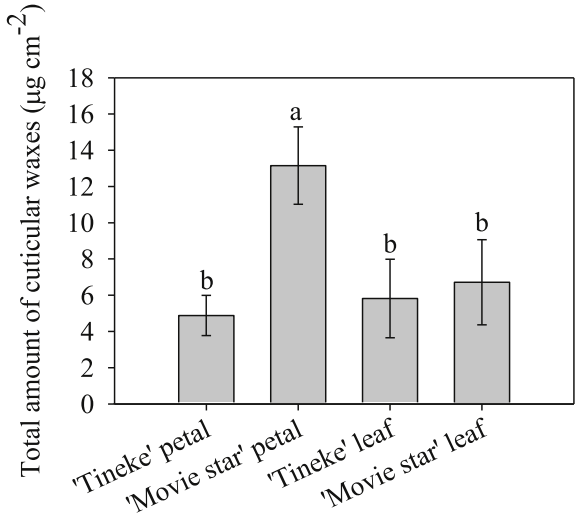

Fig. 2. Total amount of cuticular wax on the petal and leaf of *Rosa chinensis* 'Movie star' and 'Tineke'. Data are given as means ± standard deviation ($n = 5$). The different lowercase letters on the bars indicate significant differences ($p < 0.05$).

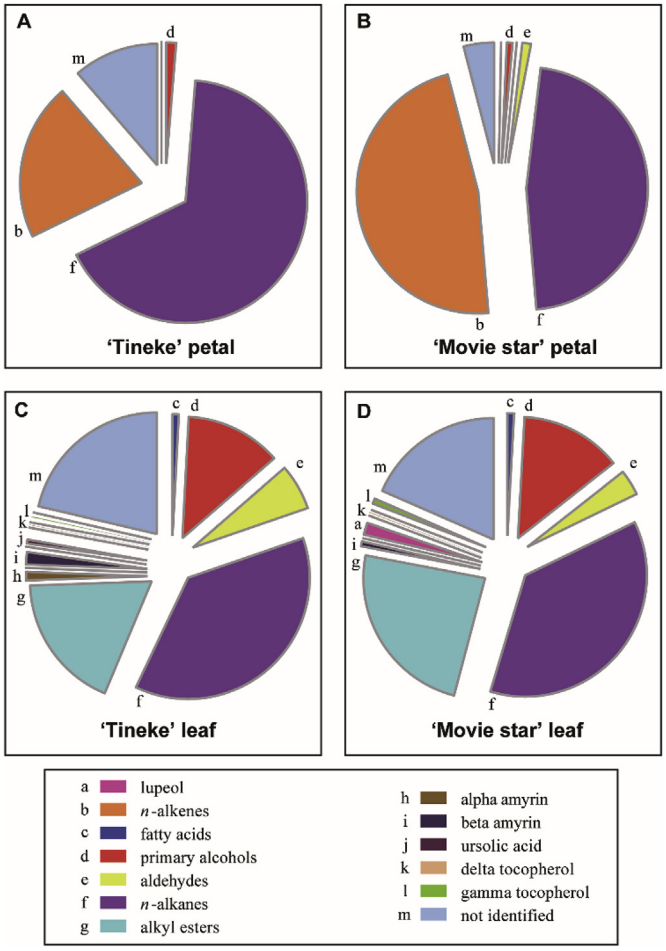

Fig. 3. Composition of cuticular wax in *Rosa chinensis* 'Tineke' (A, C) and 'Movie star' (B, D). (A, B) Petal; (C, D) leaf. Data are given as means ± standard deviation ($n = 5$).

and *n*-alkenes were found to be the major components. In the 'Movie star' petal, similar concentrations of *n*-alkanes (46.8%) and *n*-alkenes (47.3%) were detected, whereas *n*-alkanes occurred in a three-fold higher concentration (64.3%) than *n*-alkenes (20.2%) in the petals of 'Tineke' (Fig. 3A and B).

Compared to the petal wax, the amount of leaf wax varied only slightly, from 5.8 μg cm^{-2} for 'Tineke' leaves to 6.7 μg cm^{-2} for 'Movie star' leaves (Fig. 2). Similar to petal wax, the VLCFAs dominated the leaf wax mixture, including *n*-alkanes (36.8% for 'Movie star' and 37.4% for 'Tineke') and primary alcohols (13.5% and 12.8%); small amounts of fatty acids and aldehydes were detected in addition to the dominant components (Fig. 3C and D, Supporting information Fig. S3 and Table S1). Remarkably, relatively high concentrations of alkyl esters (23.9% and 18.1%) were detected in leaf waxes, which were not found in petal waxes. In addition, leaf waxes were also identified to contain a fraction of cyclic constituents, such as pentacyclic triterpenoids, steroids, and tocopherols (Fig. 3C and D).

3.3. Chain length distribution of acyclic compounds

The acyclic fraction of VLCFAs and their derivatives dominated the wax in both leaves and petals, which could be regarded as forming a homologous series. The chain-length distributions were found to be similar between compound classes within cultivars, while the predominant carbon chain lengths were substantially different between leaf and petal. Fatty acids occurred in a fairly broad range of even-numbered chain lengths (C_{20} to C_{34}) and were predominated by C_{30} in

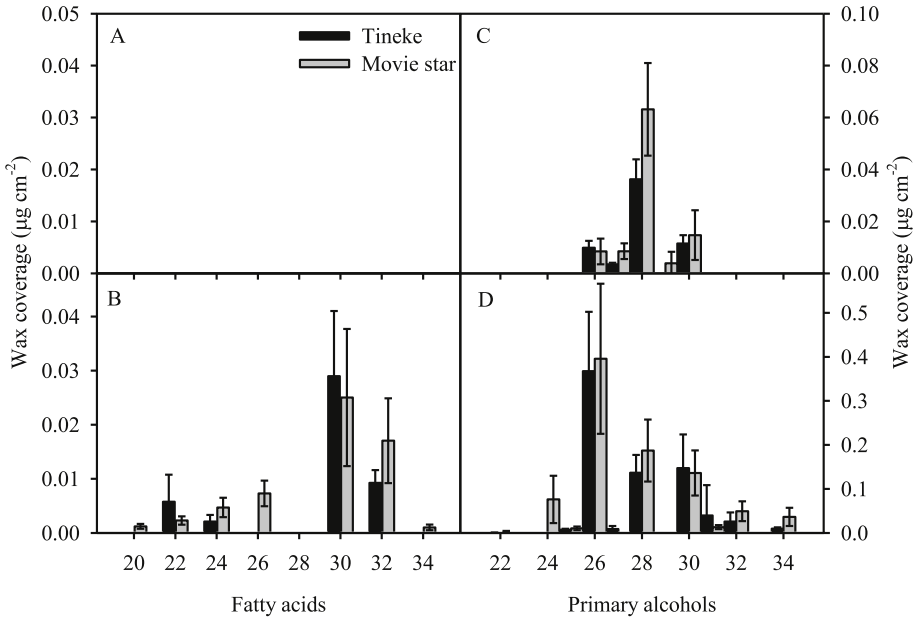

Fig. 4. Chain length distribution of fatty acids and primary alcohols in the cuticular wax of *Rosa chinensis* 'Movie star' and 'Tineke'. (A, C) Petal; (B, D) leaf. Data are given as means ± standard deviation (n = 5).

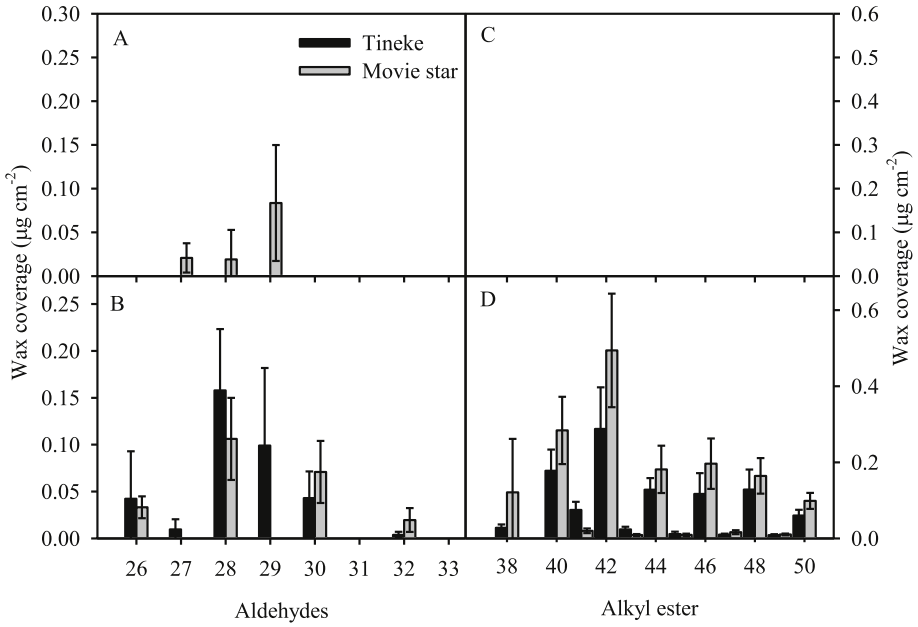

Fig. 5. Chain length distribution of aldehydes and alkyl esters in the cuticular wax of *Rosa chinensis* 'Movie star' and 'Tineke'. (A, C) Petal; (B, D) leaf. Data are given as means ± standard deviations (n = 5).

the leaf waxes of both cultivars, while only traces of or no fatty acids at all were detected in the studied petal waxes (Fig. 4). The primary alcohol fraction of leaf wax contained a range of even-numbered chain lengths from C_{22} to C_{34} and were predominated by C_{26}, whereas they ranged from C_{26} to C_{30}, with a prominent accumulation of C_{28} in petals (Fig. 4). The aldehydes ranged from C_{26} to C_{33}, with a dominant chain length of C_{28}, in leaf waxes; small amounts of aldehydes, ranging from C_{27} to C_{29}, was also found in 'Movie star' petals (Fig. 5). The unbranched alkyl esters, which were only detected in leaf waxes, ranged from C_{38} to C_{50} (Fig. 5).

The most abundant aliphatic fraction in both leaf and petal waxes consisted of *n*-alkanes. The chain lengths of the *n*-alkanes, which were predominantly odd-number carbon chains, ranged from C_{19} to C_{33}, with mostly C_{27} alkanes in petals, and from C_{25} to C_{34}, with C_{31} and C_{33} predominating, in leaves, respectively (Fig. 6). In addition, the chain lengths of unsaturated *n*-alkanes, *n*-alkenes, which were only detected in petal waxes, ranged from C_{19} to C_{33}, with C_{27} and C_{29} being present in the greatest amounts (Fig. 6).

Altogether, the weighted average chain lengths (ACLs) of acyclic compounds, which were calculated based on the molar concentration of VLCFAs, were 34.5 and 33.4 for 'Movie star' and 'Tineke' leaf waxes, respectively, whereas lower ACL values of 26.6 and 26.7, respectively, were found for petal waxes compared to that of leaves (Table 1).

4. Discussion

The present study aimed to elucidate the characteristics of the water permeability of rose petals and leaves as well as the composition of

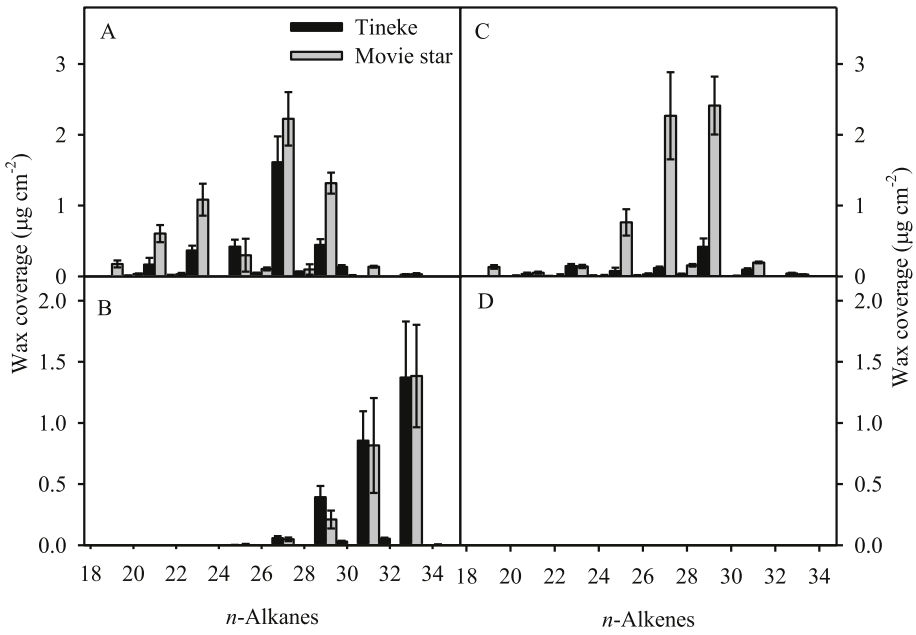

Fig. 6. Chain length distribution of *n*-alkanes and *n*-alkenes in the cuticular wax of *Rosa chinensis* 'Movie star' and 'Tineke'. (A, C) Petal; (B, D) leaf. Data are given as means ± standard deviations ($n = 5$).

their cuticular waxes as constituents of the barrier against uncontrolled water loss. As the primary function of the cuticle is considered to be the formation of a barrier to protect the aerial plant organs from excessive water loss, bulk investigations have been conducted on leaves, focusing on the properties of the cuticular layer as a transpiration barrier. The permeance for water of investigated leaves varied between 2.6×10^{-7} m s^{-1} (*Zamioculcas zamiifolia* (Lodd.) Engl.) and 4×10^{-3} m s^{-1} (*Ipomoea batatas* (L.) Lam.) after removing some of the extreme outliers; most of them were lower than 2×10^{-4} m s^{-1} (Schuster et al., 2017). In addition, a water permeability for leaves in the range of 1.0×10^{-5} m s^{-1} to 7.2×10^{-5} m s^{-1} with an overall median value of 3.6×10^{-5} m s^{-1} was found for the central 50% of the investigated species. However, to the best of our knowledge, to date, only one study has attempted to characterize water transpiration via the cuticular wax barrier in petals, in *C. bipinnatus* 'Sensation Pinkie' (Buschhaus et al., 2015). The water permeability in *C. bipinnatus* was 6.7×10^{-5} m s^{-1}, which was comparable to the range of leaf permeabilities for the central 50% of the investigated species (Schuster et al., 2017). Similar to these results, a relative high-water permeability was detected in whole petals of 'Movie star' (4.1×10^{-5} m s^{-1}) and 'Tineke' (8.9×10^{-4} m s^{-1}). Significant lower values were observed for their leaves at 1.9×10^{-5} m s^{-1} ('Movie star') and 1.8×10^{-5} m s^{-1} ('Tineke', Fig. 1).

Accordingly, the water resistance provided by the cuticular barrier for petals was less effective than that for leaves (Fig. S2). Combining this result with the data described previously for the resistance to excessive water loss in *C. bipinnatus*, the petal cuticles were found to have relatively poor barrier properties for water transpiration. This might be related to the special functions that petals have for attraction and pollination. The fast rates of transpiration for the petals is of great importance in terms of attracting pollinators, as fast transpiration rates may be accompanied by the volatilization of aroma components. This can support the hypothesis that the lower barrier properties of petals help in the faster volatilization of aroma components, which can help in the pollination of plants. Further, normally, plants flower only when environmental conditions are favourable, and petals are relatively short-lived organs. It is probably not necessary to form such a perfect transpiration barrier, compared to those of long-lived green leaves. Similar results and phenomena have been reported based on the comparative investigation of cuticular water permeability of plant species from different habitats (Schreiber and Riederer, 1996) and different organs such as fruits and leaves (Huang, 2017).

The cuticular wax of plants is considered to be the main cuticular element that contributes to the efficacy of the transpiration barrier (Riederer, 1995; Riederer and Schreiber, 2001). Thus, the cuticular waxes of both petals and leaves were comprehensively analysed. The total wax covering of the petal surfaces varied between 13.2 μg cm^{-2} ('Movie star' petals) and 4.9 μg cm^{-2} ('Tineke' petals), which falls well within the reported range for other organs like leaves and stems (Jetter and Riederer, 2016; Guo and Jetter, 2017). A variable wax load was also detected in different flower petals. The wax load of C. *bipinnatus* petals is relatively low at 2.7 μg cm^{-2} (Buschhaus et al., 2015). However, the petal wax load can be as high as 37 μg cm^{-2} in *T. officinale* petals (Guo and Jetter, 2017). In comparison to the reported total wax load in petals, the total wax load detected for leaves here is relatively low, with 5.8 μg cm^{-2} for 'Tineke' and 6.7 μg cm^{-2} for 'Movie star' (Fig. 2). A similar or higher total wax coverage was found in previous studies. The wax coverage was 10.1 and 6.2 μg cm^{-2} for 'Apollo Parade'

Table 1
Amount of very long chain acyclic fraction and cyclic fraction (μg cm^{-2}) of wax, ratio of very long chain acyclic fraction to cyclic fraction, and weighted average chain length (ACL) of the very long chain acyclic fraction of *Rosa chinensis* 'Movie star' and 'Tineke' petal and leaf. Data are given as means ± standard deviation ($n = 5$).

	Very long chain acyclic fraction	Cyclic fraction	Very long chain acyclic fraction/cyclic fraction	ACL
'Tineke' petal	4.35 ± 0.83			26.67 ± 0.17
'Movie star' petal	12.62 ± 2.13			26.59 ± 0.03
'Tineke' leaf	4.38 ± 1.72	0.20 ± 0.09	23.32 ± 7.32	33.41 ± 0.32
'Movie star' leaf	5.27 ± 1.81	0.20 ± 0.08	26.77 ± 4.54	34.47 ± 0.33

and 'Charming Parade' rose leaves (Jenks et al., 2001), respectively, and was 28 μg cm^{-2} for *R. canina* (Buschhaus et al., 2007). Therefore, the wax load for petals is also species- and cultivar-specific. In addition, the cuticular layer contributes significantly more to the overall water resistance of leaves than to that of petals. Although the total wax load for leaves and petals varies across a wide range, it could further be ascertained that there is no significant relationship between the cuticular water permeability and the total accumulation of wax (Riederer and Schreiber, 2001; Jetter and Riederer, 2016).

The wax mixtures detected in the presently investigated flower petals and leaves were highly comparable to the typical wax compositions previously reported from petals, leaves or other plant organs. A recent study on the wax of different vegetative organs in *T. officinale* found that *n*-alkanes and primary alcohols dominated the petal waxes (Guo and Jetter, 2017). In addition, in flowers of *V. faba* (Griffiths et al., 1999) and *A. majus* (Goodwin et al., 2003), relatively high concentrations of *n*-alkanes were detected. The wax mixtures on the petals of *C. bipinnatus* and *Petunia hydrida* Vilm. W115 (Petunia) differed largely from the other investigated petals, which were dominated by primary alcohols and alkyl esters, while *n*-alkanes and triterpenoids were found at low amounts (King et al., 2007; Buschhaus et al., 2015). For the 'Movie star' and 'Tineke' flowers investigated here, the wax mixtures of the petals were dominated by *n*-alkanes (46.8% and 64.3%) and *n*-alkenes (47.3% and 20.2%) (Fig. 3, Supporting information Table S1); no *n*-alkenes have been detected in other reported petals. Like in *T. officinale* flower wax (Guo and Jetter, 2017), a small amount of aldehydes was also identified in the petal wax of 'Movie star' (Fig. 3). However, several other compounds that have been reported in relatively minor amounts in leaf and petal wax mixtures of rose, such as *iso*-alkanes and *anteiso*-alkanes (Goodwin et al., 2003; Guo and Jetter, 2017), *n*-ketones (Stoianova-Ivanova et al., 1971), lactone (Hadjieva et al., 1974) and trialkyltrioxane (Mladenova et al., 1976), could not be positively identified here.

Relatively few studies so far have quantitatively or qualitatively compared the variety in the chemical composition of petal and leaf waxes. In comparison to the petal wax mixtures, the predominant VLCFAs building the wax layer of the 'Movie star' and 'Tineke' leaf cuticles, such as *n*-alkanes (36.8% and 37.4%), alkyl esters (23.9% and 18.1%) and primary alcohols (13.5% and 12.8%; Fig. 3, Supporting information Table S1 and Fig. S3), were much more diverse. Notably, higher amounts of *n*-alkenes were accumulated in the cuticle of 'Movie star' petals than in that of 'Tineke' petals. This difference might provide some distinct benefit to 'Movie star' petals. The major compound classes identified here highly matched the waxes that were detected in *R. canina* leaves (Buschhaus et al., 2007). Moreover, as in *R. canina* leaves, small amounts of fatty acids, aldehydes and pentacyclic fractions were identified in the present study. Comparison of the petal and leaf waxes for *T. officinale* at the intraspecific level showed that the wax of both organs was dominated by *n*-alkanes and branched *n*-alkanes (Guo and Jetter, 2017). *C. bipinnatus* contains high concentrations of primary alcohols in the petal wax, while *n*-alkanes and primary alcohols are the prominent components of their leaf wax (Buschhaus et al., 2015). Thus, the compositional variety of waxes may help to further develop our understanding of the physiological function of differences in the properties of the wax barrier on the surface of different plant organs.

Comparisons of the acyclic components in the wax profile of flower and leaf surfaces, in most cases, revealed a distribution of shorter chain lengths in petal waxes. The aliphatic components of *C. bipinnatus* petal wax were shown to be dominated by C_{22} and C_{24} primary alcohols and fatty acids, which were much shorter than the C_{28} and C_{30} chains of fatty acids and primary alcohols as well as the C_{29} and C_{31} chains of *n*-alkanes in its leaf waxes (Buschhaus et al., 2015). The flowers and leaves of *T. officinale* contained C_{27} to C_{31} and C_{27} to C_{33} branched and non-branched *n*-alkanes, respectively (Guo and Jetter, 2017). Similar prominent components of ester alcohols with chain lengths of C_{22} to C_{28} and C_{28} to C_{32} were found in *P. hydrida* flowers and leaves, respectively (King et al., 2007). The dominant flower wax in *Arabidopsis* was C_{29} alkane compared to C_{31} in leaves (Jenks et al., 1995; Shi et al., 2011). In the investigated 'Movie star' and 'Tineke' leaves, the predominant chain length was C_{31} and C_{33} for *n*-alkanes, C_{26} for primary alcohols, C_{30} and C_{32} for fatty acids, and C_{42} for alkyl esters (Figs. 4–6). In contrast to the chain length distribution in leaf, relative short C_{27} and C_{29} carbon chains of *n*-alkanes and *n*-alkenes as well as small amounts of C_{28} primary alcohols were detected in the petal waxes of the two cultivars (Figs. 4 and 6). These results highly matched the typical chain length distributions, ranging from C_{22} to C_{34}, for acyclic waxes on flowers or other vegetative organs (Buschhaus et al., 2015; Jetter and Riederer, 2016). As a result, the predominant pattern for flower waxes displayed shorter carbon chains than that of leaf waxes.

The variety in total wax coverage, compositional loading, and carbon chain length distribution in petals and leaves could help to determine their correlation with the transpiration barrier properties. Previous studies have attempted to address possible correlations between the total amount of wax and the properties of the transpiration barrier; however, such a correlation could not be found, based on data for leaves (Riederer and Schreiber, 2001) and fruits (Huang, 2017), either independently or in combination. Therefore, the function of cuticular wax barriers to block excessive water loss was deduced to be more likely contributed by the wax components (Jetter and Riederer, 2016). For both leaf and petal of the roses 'Movie star' and 'Tineke', the major wax components were VLCFAs. The VLCFAs are thought to be the primary elements forming the transpiration barrier, which were proposed to be tightly aligned and form obstacles in the cuticle in the form of impermeable flakes (Riederer and Schneider, 1990). As a result, molecules can only pass through the amorphous zones, which consist mainly of pentacyclic compounds such as triterpenoids and sterols (Riederer and Schneider, 1990; Riederer and Schreiber, 2001). The tight and highly ordered crystalline zones were largely related to the very-long-chain aliphatic compounds, which are thought to be the primary elements forming the transpiration barrier. For these aliphatic compounds, straight carbon chain and carbon chain-length distribute into highly ordered crystalline zones, while the branched groups or unsaturated bonds in some aliphatic compounds may largely affect the crystallinity in the wax layer (Reynhardt and Riederer, 1994). Therefore, most of the aliphatic compounds in both leaf and petal waxes were straight carbon chains and saturated. However, notably, the occurrence of unsaturated compounds, especially *n*-alkenes in petal waxes, are essentially absent in leaf waxes of the two cultivars of rose studied here. Due to the double bonds of *n*-alkenes in petal wax, the formation of highly ordered crystalline waxes at the molecular level might be significantly disturbed, thus leading to increased rates of cuticular transpiration for petals when compared to leaves.

On the other hand, the chain-length distribution of aliphatic compounds in wax layers plays an important role in barrier properties. In petals of *C. bipinnatus* 'Sensation Pinkie' (Buschhaus et al., 2015), the relatively short chain length distribution of acyclic compounds (C_{22} and C_{24} primary alcohols and fatty acids) might broaden the volume for water transportation in the wax layer, thus leading to a relatively high water permeability. Likewise, the relatively short chain lengths of the predominant VLCFA compounds in 'Movie star' and 'Tineke' were overall between C_{26} and C_{29} in petals, in contrast to those in leaves, which ranged from C_{29} to C_{33}. Consequently, the ACL was remarkably short for petals with 26.6 and 26.7, while it was 34.5 and 33.4 for leaves, respectively. The ACL has been widely used as a proxy to indicate the wax compositional quality (Poynter, 1989). The results presented here corroborate the hypothesis that a higher ACL of acyclic components might be one of the important parameters leading to the detected lower permeability in olive leaf compared to that in its fruit (Huang et al., 2017). The longer the chain length of the acyclic components is, the higher is the crystallinity of the wax layer; thus, the longer the pathway that controls water transpiration, the more effective the barrier. Therefore, the relatively short ACLs in rose petals might be

one of the main factors for the less efficient transpiration barrier properties, compared to that of their leaves.

In conclusion, the transpiration of the petals of the two rose cultivars is significantly higher than their leaves. The most abundant aliphatic compounds in the rose petals were *n*-alkanes and *n*-alkenes, whereas large amounts of primary alcohols, *n*-alkanes, alkyl esters and cyclic components were detected in leaf waxes. As a consequence, the lower ACL in petals, compared to that in leaves, could be a prominent factor leading to a higher cuticular permeability. Altogether, the presented results provide further evidence for linking cuticular chemical composition with the physiological function of blocking water transpiration and a reference for future further exploration of the properties of the cuticle as a barrier to water in different organs. Additionally, the diversity in chemical composition may also be related to special functions of petals, such as the effects of the macrostructure of surfaces on UV reflection and plant–pollinator interactions, which should be further investigated.

Contributions

HH and GPC designed and prepared the manuscript. GPC performed most of the experiments and wrote the draft of manuscript. LYZ and YJZ contributed to data analyses. SGH took part in interpretation of the results and manuscript preparation. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.01.006.

References

Burghardt, M., Riederer, M., 2003. Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. J. Exp. Bot. 54 (389), 1941–1949.
Buschhaus, C., Herz, H., Jetter, R., 2007. Chemical composition of the epicuticular and intracuticular wax layers on adaxial sides of Rosa canina leaves. Ann. Bot. 100 (7), 1557–1564.
Buschhaus, C., Hager, D., Jetter, R., 2015. Wax layers on Cosmos bipinnatus petals contribute unequally to total petal water resistance. Plant Physiol. 167 (1), 80–88.
Fich, E.A., Segerson, N.A., Rose, J.K., 2016. The plant polyester cutin: biosynthesis, structure, and biological roles. Annu. Rev. Plant Biol. 67, 207–233.
Goodwin, S.M., Kolosova, N., Kish, C.M., Wood, K.V., Dudareva, N., Jenks, M.A., 2003. Cuticle characteristics and volatile emissions of petals in Antirrhinum majus. Physiol. Plantarum 117 (3), 435–443.
Griffiths, D.W., Robertson, G.W., Shepherd, T., Ramsay, G., 1999. Epicuticular waxes and volatiles from faba bean (Vicia faba) flowers. Phytochemistry 52 (4), 607–612.
Griffiths, D.W., Robertson, G.W., Shepherd, T., Birch, A.N.E., Gordon, S.C., Woodford, J.T., 2000. A comparison of the composition of epicuticular wax from red raspberry (Rubus idaeus L.) and hawthorn (Crataegus monogyna Jacq.) flowers. Phytochemistry 55 (2), 111–116.
Guo, Y., Jetter, R., 2017. Comparative analyses of cuticular waxes on various organs of potato (Solanum tuberosum L.). J. Agric. Food Chem. 65 (19), 3926–3933.
Hadjieva, P., Stoianova-Ivanova, B., Danieli, B., 1974. Composition and structure of lactones from rose flower wax. Chem. Phys. Lipids 12 (1), 60–63.
Huang, H., 2017. Comparative Investigation of the Chemical Composition and the Water Permeability of Fruit and Leaf Cuticles. PhD thesis. Opusbibliothekuni-wuerzburgde.
Huang, H., Burghardt, M., Schuster, A.-C., Leide, J., Lara, I., Riederer, M., 2017. Chemical composition and water permeability of fruit and leaf cuticles of Olea europaea L. J. Agric. Food Chem. 65 (40), 8790–8797.
Jenks, M.A., Tuttle, H.A., Eigenbrode, S.D., Feldmann, K.A., 1995. Leaf epicuticular waxes of the eceriferum mutants in Arabidopsis. Plant Physiol. 108 (1), 369–377.
Jenks, M.A., Andersen, L., Teusink, R.S., Williams, M.H., 2001. Leaf cuticular waxes of potted rose cultivars as affected by plant development, drought and paclobutrazol treatments. Physiol. Plantarum 112 (1), 62–70.
Jetter, R., Riederer, M., 2016. Localization of the transpiration barrier in the epi-and intracuticular waxes of eight plant species: water transport resistances are associated with fatty acyl rather than alicyclic components. Plant Physiol. 170 (2), 921–934.
King, A., Nam, J.-W., Han, J., Hilliard, J., Jaworski, J.G., 2007. Cuticular wax biosynthesis in petunia petals: cloning and characterization of an alcohol-acyltransferase that synthesizes wax-esters. Planta 226 (2), 381–394.
Müller, C., Riederer, M., 2005. Plant surface properties in chemical ecology. J. Chem. Ecol. 31 (11), 2621–2651.
Martin, L.B., Rose, J.K., 2014. There's more than one way to skin a fruit: formation and functions of fruit cuticles. J. Exp. Bot. 65 (16), 4639–4651.
Mladenova, K., Stoianova-Ivanova, B., Daskalov, R., 1976. Trialkyltrioxanes in flower wax of some decorative roses. Phytochemistry 15 (3), 419–420.
Nobel, P., 2009. Physicochemical and Environmental Plant Physiology. Academic Press, Oxford.
Poynter, D., 1989. Judging the duration of time intervals: a process of remembering segments of experience. In: Advances in Psychology. Elsevier, pp. 305–331.
Reynhardt, E., Riederer, M., 1994. Structures and molecular dynamics of plant waxes. Eur. Biophys. J. 23 (1), 59–70.
Riederer, M., 1995. Waxes: the transport barriers of plant cuticles. Waxes: Chem. Mol. Biol. Funct.
Riederer, M., Schneider, G., 1990. The effect of the environment on the permeability and composition of Citrus leaf cuticles. Planta 180 (2), 154–165.
Riederer, M., Schreiber, L., 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. J. Exp. Bot. 52 (363), 2023–2032.
Samuels, L., Kunst, L., Jetter, R., 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu. Rev. Plant Biol. 59, 683–707.
Schönherr, J., 1976. Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. Planta 131 (2), 159–164.
Schreiber, L., Riederer, M., 1996. Ecophysiology of cuticular transpiration: comparative investigation of cuticular water permeability of plant species from different habitats. Oecologia 107 (4), 426–432.
Schreiber, L., Schonherr, J., 2009. Water and Solute Permeability of Plant Cuticles. Springer.
Schuster, A.-C., Burghardt, M., Riederer, M., 2017. The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions? J. Exp. Bot. 68 (19), 5271–5279.
Shi, J.X., Malitsky, S., De Oliveira, S., Branigan, C., Franke, R.B., Schreiber, L., Aharoni, A., 2011. SHINE transcription factors act redundantly to pattern the archetypal surface of Arabidopsis flower organs. PLoS Genet. 7 (5), e1001388.
Stoianova-Ivanova, B., Mladenova, K., Popow, S., 1971. The composition and structure of ketones from rose bud and rose flower waxes. Phytochemistry 10 (6), 1391–1393.
Whitney, H.M., Bennett, K.V., Dorling, M., Sandbach, L., Prince, D., Chittka, L., Glover, B.J., 2011. Why do so many petals have conical epidermal cells? Ann. Bot. 108 (4), 609–616.