Journal of Plant Physiology 167 (2010) 974–980

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Does the microclimate under hail nets influence micromorphological characteristics of apple leaves and cuticles?

Mauricio Hunsche*, Michael M. Blanke, Georg Noga

Institute of Crop Science and Resource Conservation – Horticultural Science, University of Bonn, Auf dem Huegel 6, D-53121 Bonn, Germany

ARTICLE INFO

Article history:
Received 23 December 2009
Received in revised form 4 February 2010
Accepted 4 February 2010

Keywords:
Adaxial leaf surface
Calcium
Light quality
Penetration
Wettability

ABSTRACT

A higher frequency of hail storms, possibly due to climate change, has led to increased installation of hail nets worldwide. The objective of the present work was to investigate potential effects of the microclimate under these hail nets on micromorphological characteristics of the leaves and adaxial leaf cuticles. Leaves of apple cultivars ‘Pinova’ and ‘Fuji’ grown on trees under white (highly translucent) or red-black (low transmittance) hail nets or on uncovered (control) trees were evaluated in June, August, September and October. The microclimate under the colored hail nets had no impact on leaf micromorphology, amount of cuticular wax, or leaf thickness. Similarly, no differences in thickness and permeability for calcium could be established between cuticles of leaves grown on trees under the two types of hail nets or uncovered trees. For all evaluated parameters, significant differences were detected between the two cultivars examined. In both cultivars, leaf wax synthesis followed a characteristic curve, increasing from the first to the second evaluation, and then decreasing continuously without affecting cuticular penetration of calcium. Overall, our results show that a reduction of the hail nets by 6–10% in both light and humidity was insufficient to influence the surface properties of apple leaves and permeability of cuticles. This may suggest that pest management strategies, i.e. formulation of agrochemicals, their application and dose, do not need to be adapted when used under hail nets. Overall, the present results indicate that the microclimatic changes brought about by colored hail nets are sufficient to enhance the vegetative growth and induce the ‘shade avoidance syndrome’, but do not appear to affect the leaf cuticular properties.

Introduction

In modern pomiculture, the intensive use of natural resources associated with technology is essential for maintaining elevated productivity levels and excellent fruit quality year after year. In the last decades, efforts have been made to select new cultivars, improve orchard management, optimize fertilization and crop protection strategies, while changing climatic conditions and extreme weather events such as hail constitute unpredictable and uncontrollable risks for product losses. In this context, a unique alternative for fruit growers is to passively protect their high value crops by installing hail nets over apple trees. As an additional positive effect, hail nets reduce sunburn damage on fruits, while adverse effects such as modification of plant growth and development and fruit coloration (Solomakhin and Blanke, 2008; Blanke, 2009) may also occur at the tree level. These negative aspects of hail nets have been associated with alterations of microclimate, including the intensity and quality of the transmitted light (Solomakhin and Blanke, 2009). Leaves of apple trees grown outside can synthesize up to three times more cuticular wax per surface area as compared to the same species grown in the greenhouse (Hunsche et al., 2004), suggesting a similar situation under hail nets. The plant cuticle and surface micromorphology are of special interest from an ecological and agronomical point of view. The cuticle, composed mainly of cutin, waxes, and polysaccharides, is the interface between aerial plant organs, viz leaves as well as fruits, stems, petals, sepals, and buds, and the atmosphere (Martin and Juniper, 1970). On the one hand, the cuticle prevents evaporative loss of water from the plant, but it is also the first interaction site for aerial insects, pathogens, and leaf-applied agrochemicals (Hull et al., 1975). The surface micromorphology, determined by characteristics of the cuticle, the epicuticular waxes deposited on it and trichomes, is of fundamental importance, especially in the case of pesticide adhesion and retention (Hall et al., 1997). The amount and composition of extruded waxes determine the leaf surface fine microstructure (Baker, 1982; Barthlott et al., 1998), having a great impact on surface wettability (Barnes et al., 1996; Beattie and Marcell, 2002) and cuticular penetration of externally applied products (Hunt and Baker, 1982).

Abbreviations: SEM, scanning electron microscope; PAR, photosynthetic active radiation.

* Corresponding author.
E-mail address: MHunsche@uni-bonn.de (M. Hunsche).

0176-1617/$ – see front matter
doi:10.1016/j.jplph.2010.02.007

The amount and composition of synthesized waxes and their arrangement on the surface is strongly influenced by environmental factors such as temperature (Baker, 1974; Reed and Tukey, 1982), relative humidity (Baker, 1974), and light including photosynthetically active radiation (Baker, 1974; Reed and Tukey, 1982; Takeoka et al., 1983; Cape and Percy, 1993), as well as UV-B radiation (Barnes et al., 1996; Bringe et al., 2007). Furthermore, the nutritional status of plants, such as iron deficiency, may affect the barrier properties of the leaf surface (Fernández et al., 2008). Phytohormones such as gibberellins (Knoche and Peschel, 2007) and abscisic acid (Luo et al., 2007) are also involved in co-regulating synthesis and/or extrusion of waxes from the epidermal cells to the surface.

Of all the environmental factors cited above, temperature, and light intensity and quality are strongly affected by colored hail nets, depending on their characteristics, i.e. color, thickness and number of longitudinal and transverse threads and mesh size (Blanke, 2009). Any alteration of leaf micromorphology and cuticular properties would have a great impact for plant protection activities such as adhesion and retention of sprayed agrochemicals as well as their penetration through the hydrophobic cuticular membrane. In studies at the leaf level under natural orchard conditions, we tested the hypothesis that microclimatic changes brought about by colored hail nets with modifications of temperature, relative humidity, and light conditions may influence leaf micromorphology as well as cuticular and penetration properties. This study was carried out on two new commercially important apple cultivars, 'Pinova' and 'Fuji', grown outside (control) or under white or red-black hail nets, having the highest and the lowest light transmission, respectively.

Materials and methods

Experimental setup

White (mesh size 3 mm × 9 mm) and red-black (single transverse red and longitudinal twisted double black fibers, mesh size 2.5 mm × 6.5 mm) hail nets (BayWa, Tettnang, Germany) were selected as representatives for those with a high and low light transmission in an experimental orchard at Campus Klein-Altendorf, University of Bonn, Germany during the growing seasons of 2007 and 2008. Five-year-old apple trees of cvs 'Fuji Kiku 8' and 'Pinova,' both on M9 rootstock, were sampled twice in the 2007 season and four times in 2008 on 5th June, 1st August, 2nd September and 15th October, by collecting the 5th to 7th fully expanded leaves from current-year shoots of 30 trees of each cultivar and growing condition. Apple trees of the same cultivar and orchard, but without hail net protection, served as controls and were subjected to the same pesticide management within the Integrated Fruit Program (IFP).

Preparation of leaf samples

All leaf samples were collected in the early morning and cold transported (approx. 10 °C) in plastic bags to the laboratory. Before analysis, leaf samples were washed in 3% citric acid solution (citric acid monohydrate, Sigma–Aldrich Inc., St. Louis, MO, USA) and rinsed twice in distilled and deionized water (Milli-Q Ultrapure Water, Millipore Corporation, Billerica, MA, USA) and then left to dry for 30 min under laboratory conditions.

Characterization of leaf micromorphology

The micromorphology of the collected apple leaves ($n = 5$ per sampling date and variety) was characterized by goniometry using a G10 contact angle measuring system (Krüss GmbH, Hamburg, Germany). Three disks were punched from each sampled leaf and fixed with double-sided adhesive tape (Tesa AG, Hamburg, Germany) onto glass plates. The contact angles were measured on 1 μL distilled water droplets ($n = 30$) deposited with a microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) on the apple leaf surfaces. Immediately afterwards, additional disks from the same leaves were punched out and fixed with double-sided adhesive on electrically conductive carbon planchets (Plano GmbH, Wetzlar, Germany), previously mounted on aluminum SEM stubs. Adaxial and abaxial sides of these fresh samples were examined using a scanning electron microscope (ESEM XL 30 FEI-Philips, Eindhoven, Holland) at a pressure of 0.933 mbar and acceleration beam voltage of 20 kV. A Peltier cooling element maintained the specimen temperature close to 5 °C. Transverse sections of fresh leaves and enzymatically isolated leaf cuticles were examined in a similar way to measure their respective thickness.

Determination of the amount of cuticular wax

Cuticular wax was extracted by immersing individual leaves in chloroform (Fisher Scientific UK Ltd., Leicestershire, UK) for 20 s so that both the adaxial and abaxial leaf laminas were in contact with chloroform, while leaves were gently moved with forceps. Thereafter, leaves were left to air dry for 10 min before scanning for determination of the surface area. The amount of extracted wax was determined gravimetrically after chloroform evaporation and the wax concentration was expressed as $\mu g\,cm^{-2}$ leaf area.

Isolation of cuticles and determination of cuticular permeability

After punching out 25 mm diameter leaf disks, the abaxial surface was marked to distinguish it from the adaxial surface. The abaxial surface was later discarded. Cuticular membranes were enzymatically isolated (Orgell, 1955) using cellulase ($20\,mL\,L^{-1}$ Celluclast, National Centre for Biotechnology Education, The University of Reading, Reading, UK) and pectinase ($20\,mL\,L^{-1}$ Trenolin® Flot DF, Erbsloeh Geisenheim AG, Geisenheim, Germany). After approximately 20 days, when cuticles were completely free from cell walls, cuticular membranes were rinsed with distilled water and transferred into a buffer solution for 5 days. Thereafter, adaxial cuticles were removed from the buffer solution and dried at room temperature for 2 days before dry-storing in closed Petri dishes. Before each experiment, cuticles were checked for their integrity using a stereo microscope.

The cuticular permeability was determined using the finite-dose-system by quantifying the amount of penetrated active ingredient after a predefined time (Kraemer et al., 2009a). The foliar fertilizer calcium chloride ($CaCl_2{\cdot}2H_2O$, Merck KGaA, Darmstadt, Germany) in a concentration of $10\,g\,CaCl_2\,L^{-1}$ was used as a model substance for penetration of polar substances through cuticles. Three 1 μL droplets of the solution were gently deposited on each cuticle ($n = 7$ for each treatment and sampling time) after placing them in a penetration chamber in a $0.15\,m^3$ perspex container for 3 h ($T = 26\,°C$; RH = 52%). Adaxial cuticles were then removed from the penetration chamber; the receiver solution was transferred to volumetric flasks, which were filled with 2 mL of the solution, and the calcium concentration analyzed by atomic absorption spectrometry (AAS, PerkinElmer, Analyst 300, Wellesley, USA). The percentage of penetrated calcium was calculated with respect to the applied amount.

Statistics

Data were statistically analyzed with SPSS 17 software (SPSS Inc., Chicago, IL, USA). Means were compared by analysis of variance (ANOVA, $p \leq 0.05$), considering each variety and growth condition. Wax accumulation over time was subjected to a regression analysis.