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# Parka® Has No Effects on Cuticle Deposition, Strain Relaxation, Water Movement, or Cracking of Sweet Cherry Fruit

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***Abstract.*** **Rain cracking is a severe problem for producers of sweet cherry fruit. With the aim of reducing fruit cracking, a number of different proprietary products are applied as whole canopy sprays but with variable success. Parka® is a product believed to function as a cuticle supplement. The objective of this study was to determine the effects of Parka® on cuticle deposition, strain relaxation, water movement, and fruit cracking. Cracking was quantified following simulated rain in a green house and using classical cracking assays. Fruit mass increases sigmoidally with time between 36 and 105 d after full bloom (DAFB). Cuticle synthesis and deposition—quantified as the amount of $^{13}$C-oleic acid incorporated into the cutin fraction—occurs only during stage I and II development and not thereafter. There were no effects of Parka® applied at stage I (petal fall) and at early stage III (straw yellow) on the mass of the cuticular membrane (CM) or of the dewaxed CM (DCM) in the cheek region of a range of sweet cherry cultivars including Annabella, Hedelfinger, Regina, Sam, Schneiders, and Sweetheart. In the stylar end region, the effects were variable. The wax mass per unit area was unaffected. Multiple applications of Parka® (12 times in weekly intervals beginning 14 DAFB) increased CM and DCM mass per unit area in the cheek and the stylar scar region; the effect on wax mass was significant only in the cheek region. There was no effect of Parka® on strain relaxation of the cuticle following excision of the epidermal segment and isolation of the CM. Multiple sprays of Parka® (12 times) left heavy spray deposits in all regions of the fruit surface. When applied only twice, light spray deposits were observed. With few exceptions, Parka® had no effects on water uptake flow rates or water flux densities or cracking. When whole trees were sprayed with Parka® and later exposed to simulated rain in a fog chamber, 12 Parka® applications increased cracking in one year of two, while two Parka® applications had no effect at all. Parka® sprays had no effect on the permeance of polycarbonate films to water vapor. Based on our results Parka® does not function as a cuticle supplement in sweet cherry.**

Cracking of sweet cherry fruit imposes a major limitation to production in all areas where rainfall occurs shortly before or during the harvest season. A few hours of rain may be sufficient to cause significant cracking. Fruit with macroscopic (visible) cracks is excluded from all markets. Ripe and near ripe fruit exposed to rain can remain intact (uncracked) macroscopically but can still suffer from severe microscopic (invisible) cracking (microcracking) in the cuticle (Knoche and Peschel 2006). While microcracking does not directly impair a fruit's visual quality, it does increase the incidence of fruit rots and the rate of postharvest water loss, resulting in reduced firmness and reduced sheen (Børve et al. 2000). Consequently, cracking and microcracking reduce both the quantity of marketable fruit and their quality.

To date, only rain shelters have been shown able to significantly and consistently reduce the incidence of rain cracking (Blanke et al. 2017; Cline et al. 1995). However, the installation of rain shelters (their support structures and materials) is capital intensive, while their operation (opening and closing) is labor intensive.

Spray applications of Ca salts are often reported as being effective in reducing macrocracking in sweet cherry, but the results are at best very variable. A recent review (Winkler and Knoche 2019) finds that while cracking can sometimes be significantly reduced, in many cases cracking is not reduced, despite achieving significant increases (up to 60%) in fruit Ca (Winkler et al. 2024). Although increased cross-linking is an important mechanism for Ca action in reducing cracking, Ca concentrations achieved by Ca spray applications are typically too low at the crack tip to consistently inhibit crack propagation (Winkler et al. 2024).

Thus, it is not surprising that growers are tempted to try out proprietary spray products that promise significant reductions in fruit cracking. A number of such products are on the market. All such products claim some hypothetical mode of action, but few offer robust evidence of efficacy. Most products fall in one of the following three categories:

(1) Film formers: These spray products are claimed to reduce cracking by reducing the water permeability of the fruit surface, thereby reducing water uptake (Meland et al. 2014; Torres et al. 2014). This category of compounds has a contact mode of action, so complete coverage is essential. Hence, both the fruit and the leaves will be affected. Clearly, to be effective the film must have a lower water permeance than the fruit cuticle. Also, the film must not interfere with foliar gas exchanges and so not reduce foliar photosynthesis or transpiration. Nor should the film reduce fruit transpiration. There are obvious conflicts here.

(2) Osmotically active compounds: These are presumed to reduce the rate of fruit water uptake through their rain-wetted surfaces by reducing the driving force for water uptake (Winkler et al. 2019; Wójcik et al. 2013). However, these compounds have several limitations. To be effective, they must not be washed off during rainfall. In addition, experiments demonstrate that the duration of surface wetness (hours) is more important for cracking than the amount of rainfall (mm) (Winkler et al. 2020). Finally, like the film formers, these compounds are presumed to have a contact mode of action, so complete coverage is essential.

(3) Cuticle supplements: A third category of compounds claims to decrease the water permeability of the fruit cuticle. An example is the recently introduced product Parka® (Cultiva, Las Vegas, NV, USA). Its active principle is based on fatty acids. It is argued that these supplemental fatty acids act as precursors for cutin monomers. When applied, the fatty acids are presumed to be taken up, and the resulting supply of additional precursors stimulates cutin synthesis and thus cutin deposition. We are unaware of any experimental evidence to support these claims—either for sweet cherries or for any other fruit crop species. Recent studies with apples reveal that oleic acid fed to the surface of developing apple fruitlets is indeed incorporated into the cutin polymer (Si et al. 2021a, 2021b). Thus, it may reasonably be hypothesized that feeding cutin precursors to developing sweet cherry fruit will also increase cuticle deposition. Indeed, an increase in cuticle deposition could reduce elastic strain and hence reduce microcracking and the various negative-going consequences arising therefrom (Khanal et al. 2013, 2014; Knoche and Lang 2017; Lai et al. 2016). This strategy would seem to hold potential for reducing

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macrocracking, microcracking, and fruit rot incidence (Børve et al. 2000).

The objective of our study was to determine the effects of the cuticle supplement Parka® on cuticle deposition, strain relaxation, water movement, and cracking in sweet cherry fruit.

## Materials and Methods

*Plant material.* Sweet cherry fruits of the cultivars Annabella, Hedelfinger, Regina, Sam, and Schneiders Späte Knorpel were sampled from field-grown trees grafted on 'Gisela 5' rootstocks (*Prunus cerasus* L. × *Populus canescens* Bois) at the Horticultural Research Station of the Leibniz University in Ruthe, Germany (lat. 52°14′N, long. 9°49′E). All fruits from the field were grown under a rain shelter. 'Sweetheart' sweet cherry fruit was sampled from potted trees grafted on 'Gisela 3' rootstocks and grown under a rain shelter at the Herrenhausen Campus of Leibniz University Hannover (lat. 52°27′N, long. 09°84′E). Fruit was grown according to current integrated fruit production practices (Kneib and Schulz 2006; for crop protection measures, see Supplemental file 1). Fruit were harvested at commercial maturity based on color and selected for uniformity of size and freedom from defects.

*Quantification of $^{13}$C-oleic acid incorporation into the cuticle of developing sweet cherries.* The developmental time course of cuticle deposition was determined using $^{13}$C-labeled oleic acid as a probe and the procedure described earlier (Si et al. 2021a, 2021b). Briefly, feeding solutions were prepared using uniform $^{13}$C-labeled oleic acid (>95% purity; Larodan AB, Solna, Sweden) emulsified in 0.05% aqueous nonionic surfactant (Glucopon 215 UP/Mb; BASF SE, Ludwigshafen am Rhein, Germany) at a final concentration of 167 µM (equivalent to 50 mg·L$^{-1}$). The feeding solution was vortexed for at least 3 min immediately after preparation and again a few minutes before field feeding (Si et al. 2021a).

For feeding, a 2 mL Eppendorf tube was mounted in the cheek region on the fruit surface using a fast-curing silicone rubber (Dowsil™ SE 9186 Clear Sealant; Dow Toray, Tokyo, Japan). The tube was then filled with 400 µL of the feeding solution containing $^{13}$C-oleic acid. After 24 h, the tube was removed, and the original footprint of the Eppendorf tube was marked with a permanent marker. The fruit was allowed to incorporate the absorbed $^{13}$C-oleic acid into the cutin fraction for 7 d. At 7 d after feeding, fruit were sampled, and the surface was rinsed with a surfactant solution and then carefully blotted dry with soft tissue paper. An epidermal skin segment (ES) including the original footprint of the Eppendorf tube was excised using a biopsy punch (8-mm diameter; Acuderm Inc., Fort Lauderdale, FL, USA). The cuticles were enzymatically isolated using pectinases and cellulases as described below. Previous studies have shown that this feeding system results in consistently high levels of incorporation of the labeled precursor into the cutin fraction of developing apples (Si et al. 2021b). Analysis of the wax fraction is not conclusive because a simple partitioning of the precursor into the wax fraction may cause artifacts.

The amount of C and the composition of regular carbon ($^{12}$C) and stable isotopic carbon ($^{13}$C) were quantified as described previously (Si et al. 2021b). Briefly, ∼0.25 mg of dried dewaxed cuticular membrane (DCM) discs was weighed into an aluminum pan, and the pan was crimped. Samples were combusted in the oxidation reactor of an elemental analyzer at 1080 °C under a pulse of oxygen. Combustion to $CO_2$ was catalyzed by the $CeO_2$ packing of the oxidation reactor. The C content was quantified using a thermal conductivity detector. The detector was calibrated with a commercial sediment standard for each measurement. Sucrose (IAEA-CH-6), cellulose (IAEA-CH-3), and caffeine (IAEA-600) were used as standards for C isotopic composition. An in-house standard of spruce litter was used as an internal standard for quality control of C composition and referenced isotopic composition.

The relative contribution of tracer-derived carbon ($R_{\text{Tracer}}$) (new carbon) to the total carbon pool (old plus new carbon) was calculated using a two-pool dilution model according to Gearing (1991) and the following equation:

$$R_{\text{Tracer}} = \frac{\text{at\% L} - \text{at\% C}}{\text{at\% T} - \text{at\% C}} \times 100 \qquad [1]$$

In this equation, at% T represents the atomic percentage value of tracer, and at% L and at% C represent the atomic percentage values of the labeled and unlabeled control DCM, respectively. The total mass of tracer in the whole DCM sample ($M_{\text{Tracer}}$) was calculated using the following equation:

$$M_{\text{Tracer}} = \frac{R_{\text{Tracer}} \times M_{\text{Sample}} \times \%C}{m_{\text{Sample}}} \qquad [2]$$

where $M_{\text{Sample}}$ is the total mass of sample used for the labeling procedure, %C is the carbon content of the sample, and $m_{\text{Sample}}$ is the molar mass of C in the sample. All percentage values used in the above equations were divided by 100 before calculation. Using these equations, the amounts of uptake and incorporation of $^{13}$C-oleic acid into the cutin fraction were quantified in developing sweet cherries.

*Spray application.* Parka® was applied to trees with a hand-held sprayer at the recommended dose rate (1%; Cultiva) until runoff either twice at petal fall and straw yellow (according to the manufacturer's specifications) or 12 times (at weekly intervals) beginning at petal fall, ∼14 d after full bloom (DAFB). The developmental stages of the two applications recommended by the manufacturer correspond to stage I (petal fall) and the stage II/III transition (straw yellow) (Lilleland and Newsome 1934). Unsprayed trees were used as controls. Fruit were harvested at commercial maturity based on color and size and processed on the same day. Spray deposits were viewed after 2 or 12 applications of Parka® in 'Sweetheart'. Fruit were incubated in a solution of the fluorescence tracer acridine orange (0.1%) for 10 min, removed from solution, rinsed with deionized water, blotted dry using soft tissue paper, and viewed under a fluorescent microscope [MZ10F; Leica Microsystems, Wetzlar, Germany; filter GFP plus, 440- to 480-nm excitation wavelength, ≥510-nm emission wavelength (Leica); camera DP 73 (Olympus, Tokyo, Japan)]. The number of individual fruit replicates was ten. The surface area covered by a single spray of Parka® at the recommended dose rate was quantified using the tracer calcofluor white at 0.01% in 'Sam'. Briefly, fruit were sampled, mounted on a drying rack using clothespins, and sprayed once to runoff. Following drying, fruit were transferred to the stage of the microscope (MZ10F; Leica). Stem cavity, stylar end, cheek, and suture were viewed in incident ultraviolet light [filter ultraviolet, 360- to 440-nm excitation wavelength, ≥420-emission wavelength (Leica); camera DP73 (Olympus)], and the fluorescing area was quantified (cellSens 1.7.1; Olympus Soft Imaging Solutions, Müenster, Germany). The number of individual fruit replicates was ten. The effects of Parka® on cuticle deposition, strain relaxation, water uptake, and cracking were quantified.

*Cuticle isolation, cuticle mass per unit area, and strain relaxation.* The effect of two applications of Parka® on cuticle deposition was evaluated in 'Annabella', 'Hedelfinger', 'Regina', 'Sam', 'Schneiders Späte Knorpel', and 'Sweetheart'. In an additional experiment, 'Sweetheart' trees were also sprayed at weekly intervals. Fruits were harvested, ES was excised using a biopsy punch (8 mm; Acuderm), and cuticular membranes (CMs) were isolated using pectinases and cellulases. For cuticle isolation, the ES were incubated in 50 mM citric acid buffer (at pH 4, adjusted using NaOH) containing pectinase (90 mL·L$^{-1}$; Panzym Super E flüssig, Novozymes A/S, Krogshøjvej, Bagsværd, Denmark) and cellulase (5 mL·L$^{-1}$; Cellubrix; Novozymes A/S) (Orgell 1955; Yamada et al. 1964). Lastly, $NaN_3$ was added at a final concentration of 30 mM to inhibit microbial growth. After the CM had separated from the flesh, the CM was carefully cleaned from adhering cellular debris using a camel-hair brush and thoroughly rinsed in deionized water. The isolated CMs were transferred onto polytetrafluoroethylene discs for drying, held above dry silica gel for a minimum of 24 h, and then weighed on a microbalance (CPA2P, accuracy 0.001 mg; Sartorius, Göttingen, Germany). Mass per unit area was calculated. Wax was extracted by incubating CMs in methanol:chloroform (1:1 v/v) for at least 7 d. The mass per unit surface area of CM and DCM was determined by weighing CM and DCM discs on a microbalance (CPA2P; Sartorius). The discs were dried above silica gel for about 1 week and then weighed. Mass per unit area was calculated.

Isolation of the CM and determination of the apparent biaxial strain relaxation [ε (%)] following excision of the ES were carried out using the procedure of Lai et al. (2016). Briefly, the hydrated CMs were spread on a microscope slide and viewed under a microscope (Leica MZ10F; Leica). A calibrated image was taken (DP73; Olympus), and the area of the

flattened CM discs was quantified using image analysis (cellSens 1.7.1; Olympus). Strain relaxation [ε (%)] was calculated using the following equation:

$$\varepsilon(\%) = \frac{A_0 - A_{CM}}{A_{CM}} \times 100 \qquad [3]$$

In this equation, the value $A_0$ represents the cross-sectional area of the biopsy punch and $A_{CM}$ represents the area of the relaxed, isolated CM disc.

*Water uptake and cracking in immersion assays.* The effect of Parka® (Cultiva) on water uptake was studied in 'Annabella', 'Hedelfinger', 'Regina', 'Sam', and 'Schneiders'. Fruit were harvested, the pedicel was cut flush with the receptacle, and the cut end was sealed with silicone rubber. This procedure restricts water uptake to the fruit surface (Beyer et al. 2002). Fruit were incubated individually in deionized water, removed from water at regular intervals, blotted dry using soft tissue paper, weighed, and returned to the water (Beyer and Knoche 2002). Flow rates of water uptake ($F$ in kg·s$^{-1}$) were calculated on an individual fruit basis by fitting a linear regression line through a plot of cumulative water uptake vs. time. The uptake rate equals the slope of the regression line. Flux densities ($J$ in kg·m$^{-2}$·s$^{-1}$) were calculated by dividing the flow rates by the fruit surface area [$A$ (m$^2$)]. The latter was estimated from fruit mass assuming a spherical shape and a density of 1 kg·L$^{-1}$ as first approximations.

$$J = \frac{F}{A} \qquad [4]$$

The effect of Parka® on fruit cracking was investigated. Two groups of 25 fruit per treatment were incubated in deionized water. Fruit were removed from the solution at 0, 2, 4, 6, 10, and 24 h and checked for macroscopically visible cracks. Uncracked fruit were reincubated. Cracked fruit was removed from solution. The time to 50% cracking ($T_{50}$) was calculated from sigmoidal regression models fitted through plots of cracking (%) vs. time (Winkler et al. 2015).

*Cracking in artificial rain.* Cracking was also assessed under artificial rain in the greenhouse using potted 'Sweetheart' trees. Trees were treated twice with Parka® according to the manufacturer's recommendations or multiple times at weekly intervals (12 ×). At maturity, trees were exposed to artificial rain in a fog chamber as described previously (Winkler et al. 2020). Briefly, the fog was generated in a closed greenhouse compartment using deionized water and a high-pressure pump (model SS1B1511; S. Ilario d'Enza, Interpump Group, Italy; operated at 10 MPa). The system was operated intermittently for 5 s every 2 min for up to 48 h. Using these settings a dense fog was generated that wetted leaf and fruit surfaces continuously (Winkler et al. 2020). After 48 h of "rain," the number of cracked fruit per tree was counted. The number of individual tree replicates was five.

*Effect on water vapor permeance.* To establish the effect of Parka® on water vapor transport, a model system comprising polycarbonate sheets (Pokalon N38 20 µm; LONZA Folien, Weil, Germany) coated with Parka® (Cultiva) and stainless steel diffusion cells was used (Geyer and Schönherr 1988; Knoche et al. 2000). This model system was chosen, because the polycarbonate sheets are of uniform thickness and permeance to water vapor. The water vapor permeance of the polycarbonate sheet at 20 µm thickness is of the same order of magnitude as that of sweet cherry fruit skin (Knoche et al. 2000). The Parka® films were cast on polycarbobanate sheets and allowed to dry. Run-off of solution from the sheet was prevented using polyethylene (PE) rings cut from a PE pipe of 120-mm diameter. The rings were sealed to the sheets using silicone rubber (3140 RTV Coating; Dow Toray). A 10 mL aliquot of a 1% Parka® solution was pipetted into the ring and allowed to dry above dry silica gel. After the solution had dried, discs were punched from the film using a biopsy punch. Film thickness varied within the ring. However, within the small area of a 12-mm-diameter disc, the thickness of the Parka® film was uniform as judged visually from the transparency of the film. Thickness was determined by weighing discs and then expressed as a gravimetric thickness in mass per unit area. The coated discs were mounted on stainless steel diffusion cells using a high-vacuum grease (Korasilon-Paste; Kurt Obermeier, Bad Berleburg, Germany). Uncoated discs served as control. Care was taken that no grease was present on the film exposed in the orifice of the diffusion cell. The diffusion cells were filled with deionized water through a port in the base. The port was sealed with clear transparent tape that had a water vapor permeance 2 orders of magnitude lower than the polycarbonate discs (tesa 57372 Film®; Tesa-Werke Offenburg, Offenburg, Germany). This limited water loss to the coated film exposed in the orifice (7-mm diameter) of the diffusion cell. The cells were positioned upside down on a stainless-steel grid above dry silica gel in a PE box so that the film faced the silica gel. For details, see Fig. 1A in Knoche et al. (2000). The next day, the diffusion experiment was initiated. Diffusion cells were weighed at 24-h intervals during a period of 96 h (ME235P; Sartorius). Following weighing, diffusion cells were returned to the PE box. The rate of water loss ($F$ in kg·s$^{-1}$) was determined on an individual diffusion cell basis by fitting a linear regression line through a plot of diffusion cell mass vs. time. The slope of this line equaled the flow rate of water vapor through the coated disc. The permeance ($P$ in m·s$^{-1}$) of the coated discs to water vapor was calculated from:

$$P = \frac{F}{A \cdot \Delta C} \qquad [5]$$

In this equation, $A$ (m$^2$) equals the area of the orifice of the diffusion cell and $\Delta C$ (kg·m$^{-3}$) the gradient in water vapor concentration between the inside of the diffusion cell ($C_i$) and the atmosphere in the container ($C_o$) or $\Delta C = C_i - C_o$. Since the $C_o$ above dry silica gel is practically 0 (Geyer and Schönherr 1988), the driving force for transpiration is close to $C_i$. The $C_i$ equals the water vapor concentration at saturation (19.44 g·m$^{-3}$ at 22 °C) (Nobel 1999).

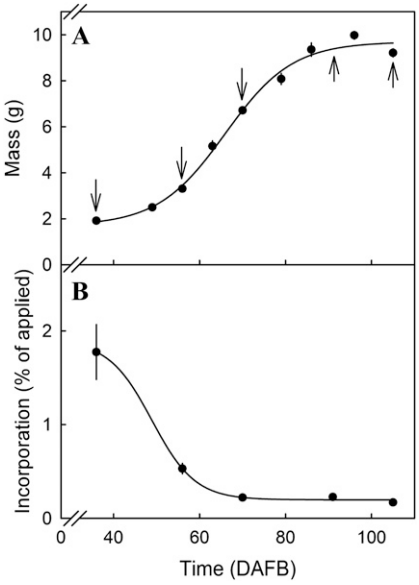


Fig. 1. Developmental time course of the change in fruit mass (**A**) and the incorporation of $^{13}$C-labeled oleic acid (**B**) into the cutin fraction of the sweet cherry fruit cuticle. Arrows indicate time of $^{13}$C application. The $^{13}$C-oleic acid was fed to the fruit using an Eppendorf tube mounted on the fruit using a fast-curing silicone rubber. The tubes were filled with $^{13}$C-oleic acid. After 24 h, the tubes were removed. At 7 d after feeding fruit were harvested, the cuticles were isolated, and the $^{13}$C-enrichment in the cutin fraction was determined.

*Data analysis.* The data are presented as mean ± standard error (*SE*). Analysis of variance and regression were conducted using the statistics software package SAS (version 9.1.3; SAS Institute, Cary, NC, USA) and R software (R 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). The means were compared using Tukey's Studentized range test ($P < 0.05$). The significance of the coefficients of determination at the 0.05, 0.01, and 0.001 levels are indicated by *, **, and ***, respectively.

## Results

During stage II and III of development, fruit increased in mass sigmoidally (Fig. 1A). Cuticle synthesis and deposition as indexed by the incorporation of $^{13}$C-oleic acid into the cutin fraction occurred only at 40 DAFB (1.5%) and 58 DAFB (0.5%) (Fig. 1B). There was little incorporation (<0.2%) thereafter. The incorporation of $^{13}$C-oleic acid did not lead to a gravimetrically detectable increase in cuticle mass per unit area.

Fluorescence microscopy of the fruit surface revealed circular microcracks in the pedicel cavity around the pedicel:fruit junction and around the scar at the stylar end of the fruit irrespective of the number of Parka® applications. These microcracks were infiltrated

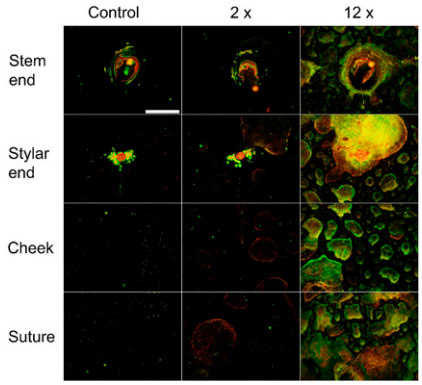


Fig. 2. Fluorescent micrographs of microcracks and Parka® spray residues in the regions of the pedicel cavity, the stylar end, the suture, and the cheek of sweet cherry fruit. Fruit were sprayed with Parka® twice according to manufacturer's recommendations (at petal fall and straw yellow) (2 ×) or weekly starting at petal fall (12 ×). Untreated fruit served as control (left column). Fruit were stained by incubation in 0.1% acridine orange for 10 min. Scale bar = 5 mm.

by the fluorescent tracer acridine orange (Fig. 2). Fruit that were sprayed weekly with Parka® had heavy spray deposits in all regions of the fruit surface. When applied twice, only light deposits were observed.

These deposits represented footprints of individual spray droplets that had dried down (Fig. 3). Within these droplet deposits, the distribution of the Parka® residue was uneven. The residues always accumulated most at the periphery of the original droplet footprint. The surface area in contact with Parka® deposits was largest in the pedicel cavity region, followed by suture region, stylar scar region, and the cheek region (Fig. 3).

There was no effect of Parka® applied at petal fall or at straw yellow on CM or DCM mass per unit area in the cheek region in any of a small range of sweet cherry cultivars (Table 1). The only exception was 'Annabella', in which CM mass was increased in the stylar region. The effect of Parka® on the DCM mass in the stylar end region was inconsistent. Occasionally, DCM mass was decreased ('Hedelfinger' and 'Sam'), increased ('Anabella' and 'Schneiders Späte Knorpel'), or unaffected ('Regina'). There was no effect on wax mass per unit area.

Multiple applications of Parka® (12 ×) increased the CM and DCM mass per unit area in the cheek and the stylar scar regions, and the effect on wax mass was significant only in the cheek region. When Parka® was applied twice, the mass of CM and DCM increased only in the cheek region, but not in the stylar end region (Table 2). The wax mass remained unchanged.

There was no effect of Parka® on strain relaxation following excision of the ES and isolation of the CM (Table 3). The only exceptions were 'Anabella' and the stylar scar region of 'Regina' and 'Sam', in which Parka® reduced strain relaxation. In the cheek region of 'Sam', strain relaxation was increased by Parka®.

Averaged across all cultivars, two applications of Parka® significantly decreased fruit mass (Table 4). Generally, Parka® had no effect on flow rates or on flux densities of water uptake (Table 4). However, this was not consistent for all cultivars. In 'Annabella', flow rates and fluxes increased, but in 'Hedelfinger', they decreased. With the exception of 'Annabella', Parka® had no significant effect on cracking as indexed by the time to 50% cracking ($T_{50}$) in an immersion assay (Table 5).

Two or multiple applications of Parka® decreased fruit mass but had no effect on rates of water uptake or flux densities as compared with two applications or to the nontreated control (Table 5). Again, there was no effect of Parka® on cracking in an immersion assay (Table 5).

When spraying fruit and exposing entire trees in a rain shelter to simulated rain, weekly applications of Parka® increased cracking in one of two seasons. When applying Parka® twice according to the manufacturer's recommendations, there was no effect on cracking in simulated rain (Table 6). Parka® had no effect on the permeability of a polycarbonate film to water vapor (Fig. 4).

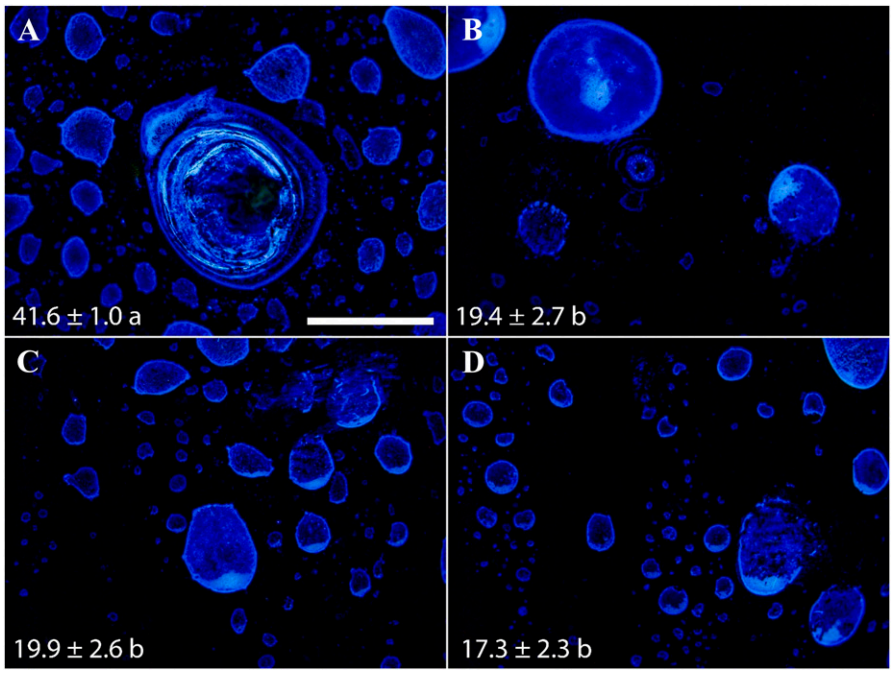


Fig. 3. Spray residues of Parka® on a 'Sam' sweet cherry. Free hanging fruit were sprayed to runoff, allowed to dry, and then viewed under a fluorescence binocular in ultraviolet incident light. Calcofluor white was used as a tracer. (**A**) Pedicel cavity region. (**B**) Stylar scar region. (**C**) Suture region. (**D**) Cheek region. Numbers indicate means and standard errors of the percentage of the fruit surface covered by spray residue in the different regions of the fruit. Means followed by the same letter do not differ significantly at $P \leq 0.05$. Scale bar = 5 mm.

## Discussion

*Cuticle deposition.* There was no effect of Parka® on cuticle deposition. We judge that the occasional increase in cuticle mass per unit area is likely an artifact caused by spray residues remaining on the cuticle surface after enzymatic isolation and wax extraction.

First, our micrographs revealed heavy Parka® deposits in the pedicel cavity region but less in the cheek and suture regions. Deposits in the stylar scar region were somewhat variable (Figs. 2 and 3). This observation finds its explanation in the behavior of the liquid spray solution. Cherries hang in the canopy, stalk up and stylar scar down, so most liquid spray runs off from (vertical) surfaces, whereas it tends to collect in a "puddle" in the (upward-facing) pedicel cavity. The suspended droplet around the (downward-facing) stylar scar either falls off or remains and dries. As seen in our micrographs, this results in heavy spray deposits in the pedicel cavity and stylar scar regions as the spray solution dries, whereas the cheek and suture regions remain largely free of visible deposits.

Second, as seen in this and earlier studies, cuticle deposition ceases during stage II (Knoche et al. 2004; Peschel et al. 2007). The cessation of cuticle deposition is the result of a downregulation of the genes involved in the synthesis of cutin and wax and so in the deposition of new CM material during stage II (Alkio et al. 2012). In the manufacturer's recommended spray scheme, only the first of the two Parka® applications, and only the first four of the twelve Parka® applications, occurred during the phase of active CM deposition. Most Parka® applications were too late, coming after CM deposition had ceased. Clearly, any precursor delivered after the genes involved in CM synthesis had been downregulated would be ineffective. There is no genetic variation in the cuticle deposition pattern, and hence, our findings are likely to not only apply to the sweet cherry cultivars investigated

Table 1. Effect of Parka® on the mass of the cuticular membrane (CM), dewaxed CM (DCM), and wax content of samples isolated from the cheek or stylar scar areas of sweet cherry fruit.

| | | Mass ($g \cdot m^{-2}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | CM | | DCM | | Wax | |
| Cultivar | Position | Control | Parka® | Control | Parka® | Control | Parka® |
| Annabella | Cheek | 1.00 ± 0.03 | 1.25 ± 0.04 | 0.80 ± 0.01 | 0.99 ± 0.02 | 0.21 ± 0.03 | 0.26 ± 0.05 |
| | Stylar scar | 2.07 ± 0.04 | 2.57 ± 0.04[i] | 1.67 ± 0.01 | 2.08 ± 0.01[i] | 0.40 ± 0.04 | 0.49 ± 0.05 |
| Hedelfinger | Cheek | 1.24 ± 0.05 | 1.20 ± 0.06 | 0.98 ± 0.01 | 0.95 ± 0.03 | 0.26 ± 0.05 | 0.25 ± 0.07 |
| | Stylar scar | 2.12 ± 0.06 | 2.00 ± 0.05 | 1.79 ± 0.02 | 1.65 ± 0.01[i] | 0.33 ± 0.07 | 0.34 ± 0.05 |
| Regina | Cheek | 1.48 ± 0.05 | 1.63 ± 0.05 | 1.10 ± 0.01 | 1.22 ± 0.04 | 0.38 ± 0.05 | 0.41 ± 0.06 |
| | Stylar scar | 2.91 ± 0.08 | 3.02 ± 0.05 | 2.35 ± 0.02 | 2.38 ± 0.02 | 0.56 ± 0.08 | 0.64 ± 0.06 |
| Sam | Cheek | 1.21 ± 0.04 | 1.27 ± 0.04 | 0.96 ± 0.00 | 1.03 ± 0.01[i] | 0.25 ± 0.04 | 0.24 ± 0.04 |
| | Stylar scar | 2.24 ± 0.10 | 2.09 ± 0.07 | 1.99 ± 0.03 | 1.71 ± 0.00[i] | 0.24 ± 0.11 | 0.39 ± 0.07 |
| Schneiders | Cheek | 1.33 ± 0.06 | 1.22 ± 0.06 | 0.99 ± 0.03 | 0.92 ± 0.02 | 0.34 ± 0.06 | 0.30 ± 0.07 |
| | Stylar scar | 2.26 ± 0.04 | 2.41 ± 0.08 | 1.84 ± 0.01 | 1.94 ± 0.02[i] | 0.42 ± 0.05 | 0.48 ± 0.08 |
| Mean | Cheek | 1.25 ± 0.11 | 1.31 ± 0.12 | 0.97 ± 0.03 | 1.02 ± 0.06 | 0.29 ± 0.11 | 0.29 ± 0.13 |
| | Stylar scar | 2.32 ± 0.16 | 2.42 ± 0.13 | 1.93 ± 0.04 | 1.95 ± 0.03 | 0.39 ± 0.16 | 0.47 ± 0.14 |

[i] Within rows, these means are significantly different from the control (Tukey's Studentized range test at $P \leq 0.05$).
Fruit were sprayed twice with Parka® according to the manufacturer's recommendations (at petal fall and straw yellow). Untreated fruit served as control. Data points represent means ± standard errors.

herein but to sweet cherry in general (Peschel and Knoche 2012). Furthermore, a modified application scheme that focuses on early applications during the phase of active CM deposition will also not be effective. Four of the twelve Parka® applications were made during this phase but also had no effect.

Third, there was no effect of Parka® on strain relaxation in the CM following excision and isolation. The lack of an effect on CM strain relaxation is consistent with the lack of an effect on CM deposition. Only if CM deposition had increased would strain relaxation have decreased (Khanal et al. 2014). Since cuticle strain is causal in microcracking, Parka® also had no effect on microcracking of the cuticle. Indeed, microcracking was occasionally found to have increased as a result of the application of multiple Parka® sprays, which inevitably increased the duration of surface wetness as these sprays were each applied to runoff.

Fourth, the occasional decrease in fruit size due to Parka is expected to result in a less strained and, hence, thicker cuticle. This, however, is an artifact. Fruit size and cuticle thickness are negatively related. These results demonstrate that Parka® does not serve to supplement cuticle development.

*Effect on water uptake and cracking.* There was no evidence for a consistent effect of Parka® on water uptake or transpiration. The water uptake flux and the transpiration permeance were unaltered by applications of Parka®. Indeed, Parka® sprays occasionally increased fruit water uptake, compared with the unsprayed control. The most likely explanation for the water flow increases is increased microcracking resulting from increased durations of surface wetness associated with the multiple Parka® sprays. It is well known that surface wetness increases microcracking of a strained cuticle, probably as a result of altered mechanical properties of the hydrated CM (Knoche and Peschel 2006). This, in turn, can cause microcracking, which impairs the barrier function of the cuticle with respect to water movements (Knoche and Peschel 2006).

Fitting with the lack of a Parka® effect on cuticle deposition, water uptake, and transpiration is the lack any consistent effects on cracking, as indexed by the time to 50% cracking ($T_{50}$). Similarly, in blueberries (*Vaccinium corymbosum*), no effects of Parka® were found for the various fruit quality attributes or for cracking (Vance and Strik 2018). Also, the effects of Parka® on cracking of Jujube (*Ziziphus jujuba* Mill.) fruit were only marginal: a <4% to 5% reduction in cracking (Ozturk et al. 2018). Our findings differ from a study by Measham et al. (2020) in sweet cherry reporting decreased cracking following applications of Parka®. Unfortunately, the mechanistic basis of the effect reported by Measham et al. (2020) is unknown. However, increased cuticle deposition must be excluded based on our current findings. Also, a mode of action for Parka® as a film former would seem highly unlikely.

First, film forming is a contact mode of action and ideally requires complete coverage with a Parka® deposit, as only the portion of the fruit surface in contact with a spray deposit would be affected. Based on our results, this contact area would be small [range, 41.6 to 17.3%) (Fig. 3), with only about 22% of the total fruit surface] with the remaining surface (78%) not being in contact. A similar low coverage (18%) was reported earlier (Knoche and Winkler 2017).

Second, water uptake into sweet cherry occurs by viscous flow through polar pathways (Beyer et al. 2005; Weichert and Knoche 2006a). Unless these pathways are somehow 'plugged' by Parka®, there will be no effect

Table 2. Effect of Parka® on the mass of the cuticular membrane (CM), dewaxed CM (DCM), and wax content of samples isolated from the cheek or stylar scar areas of 'Sweetheart' sweet cherry fruit.

| | | Mass ($g \cdot m^{-2}$) | | |
|---|---|---|---|---|
| Position | Treatment | CM | DCM | Wax |
| Cheek | Control | 0.87 ± 0.02 c[i] | 0.67 ± 0.02 c | 0.20 ± 0.02 b |
| | 2 × | 1.06 ± 0.04 b | 0.84 ± 0.03 b | 0.22 ± 0.05 b |
| | 12 × | 1.51 ± 0.05 a | 1.05 ± 0.03 a | 0.46 ± 0.06 a |
| Stylar scar | Control | 1.87 ± 0.08 b | 1.65 ± 0.08 b | 0.22 ± 0.11 a |
| | 2 × | 1.92 ± 0.06 b | 1.74 ± 0.07 b | 0.19 ± 0.09 a |
| | 12 × | 2.95 ± 0.19 a | 2.47 ± 0.17 a | 0.49 ± 0.25 a |

[i] Within positions and within columns, means followed by the same letter are not significantly different (Tukey's Studentized range test, $P \leq 0.05$).
Fruit were sprayed with Parka® twice (2 ×) at petal fall and straw yellow or 12 times (12 ×) starting at petal fall. Untreated fruit served as control. Data points represent means ± standard errors.

Table 3. Effect of Parka® on strain relaxation of cuticle samples on excision and on isolation.

| | | Strain (%) | |
|---|---|---|---|
| Cultivar | Position | Control | 2 × |
| Annabella | Cheek | 33.8 ± 1.1 | 22.6 ± 2.2[i] |
| | Stylar scar | 34.8 ± 0.9 | 18.2 ± 1.3[i] |
| Hedelfinger | Cheek | 45.8 ± 1.0 | 46.3 ± 1.1 |
| | Stylar scar | 41.0 ± 0.8 | 42.3 ± 1.3 |
| Regina | Cheek | 32.7 ± 1.3 | 29.6 ± 1.2 |
| | Stylar scar | 34.5 ± 0.9 | 30.2 ± 1.1[i] |
| Sam | Cheek | 34.5 ± 1.5 | 39.8 ± 1.3[i] |
| | Stylar scar | 37.2 ± 1.2 | 32.6 ± 1.1[i] |
| Schneiders | Cheek | 37.4 ± 1.6 | 37.7 ± 1.6 |
| | Stylar scar | 39.4 ± 1.2 | 37.9 ± 0.9 |
| Mean | Cheek | 36.8 ± 3.0 | 35.2 ± 3.4 |
| | Stylar scar | 37.4 ± 2.3 | 32.2 ± 2.6[i] |

[i] Within rows, these means are significantly different from the control (Tukey's Studentized range test, $P \leq 0.05$).
The cuticles were excised from the cheek or stylar scar regions of sweet cherry fruit using a biopsy punch. Fruit were sprayed twice with Parka® according to manufacturer's recommendations (at petal fall and straw yellow). Untreated fruit served as control. Data points represent means ± standard errors.

Table 4. Effects of Parka® on fruit mass, on water uptake rate, flux density, and on cracking of different sweet cherry cultivars.

| | Mass (g) | | Rate of water uptake ($mg \cdot h^{-1}$) | | Flux density ($\times 10^4$ $kg \cdot m^{-2} \cdot s^{-1}$) | | $T_{50}$ (h) | |
|---|---|---|---|---|---|---|---|---|
| Cultivar | Control | 2 × | Control | 2 × | Control | 2 × | Control | 2 × |
| Annabella | 6.9 ± 0.3 | 4.3 ± 0.1[i] | 3.4 ± 0.3 | 13.8 ± 3.0[i] | 5.3 ± 0.4 | 29.9 ± 6.4[i] | — | — |
| Hedelfinger | 9.0 ± 0.2 | 8.3 ± 0.2[i] | 17.8 ± 1.1 | 10.1 ± 0.8[i] | 23.6 ± 1.4 | 14.1 ± 1.1[i] | 8.8 ± 1.2 | 10.2 ± 2.6 |
| Regina | 12.1 ± 0.4 | 10.8 ± 0.4[i] | 9.3 ± 0.9 | 9.4 ± 1.0 | 10.2 ± 1.0 | 10.9 ± 1.0 | — | — |
| Sam | 9.8 ± 0.4 | 8.1 ± 0.2[i] | 7.7 ± 0.9 | 6.3 ± 0.6 | 9.5 ± 1.0 | 8.9 ± 0.8 | 3.5 ± 0.2 | 2.8 ± 0.6 |
| Schneiders | 10.2 ± 0.5 | 9.9 ± 0.4 | 6.4 ± 0.6 | 5.9 ± 0.6 | 7.8 ± 0.7 | 7.3 ± 0.7 | 4.1 ± 0.5 | 4.5 ± 0.4 |
| Mean | 9.6 ± 0.8 | 8.3 ± 0.6[i] | 8.9 ± 1.8 | 9.1 ± 3.4 | 11.3 ± 2.1 | 13.5 ± 6.0 | 5.4 ± 1.4 | 5.8 ± 2.7 |

[i]Means within rows followed by an asterisk are significantly different from the control (Tukey's Studentized range test, $P \leq 0.05$). All other means are nonsignificant.

Cracking was quantified in an incubation assay in deionized water as the time to 50% cracking ($T_{50}$). Parka® was applied twice according to manufacturer's recommendations (at petal fall and straw yellow). Untreated fruit served as control. Data points represent means ± standard errors.

Table 5. Effect of Parka® on fruit mass and on water uptake rate with 'Sweetheart' sweet cherry fruit.

| Treatment | Mass (g) | Water uptake rate ($mg \cdot h^{-1}$) | Water uptake flux ($\times 10^{-6}$ $kg \cdot m^{-2} \cdot s^{-1}$) | $T_{50}$ (h) |
|---|---|---|---|---|
| Control | 9.9 ± 0.1 a[i] | 4.8 ± 0.7 ab | 6.0 ± 0.9 a | 11.9 ± 0.7 a |
| 2 × | 9.4 ± 0.2 b | 8.6 ± 1.7 a | 9.2 ± 1.5 a | 12.7 ± 0.5 a |
| 12 × | 7.2 ± 0.1 c | 4.2 ± 1.2 b | 6.4 ± 1.7 a | 10.4 ± 0.6 a |

[i]Mean separation within columns by Tukey's Studentized range test ($P \leq 0.05$).

Parka® was applied following manufacturer's recommendation (at petal fall and straw yellow stage, 2 ×) or at weekly intervals (12 ×) starting at petal fall. Fruit were sprayed to runoff using a hand sprayer. Untreated fruit served as control. Data points represent means ± standard errors.

on water uptake. Plugging of a polar pathway results in an instantaneous reduction in water uptake (Weichert and Knoche 2006b). There is, however, no experimental evidence for plugging occurring with Parka®.

Third, our results also demonstrate that Parka® does not have anti-transpirant properties; i.e., that decrease the permeance of an intact fruit cuticle. Even a coating of Parka® two to three times as thick as the cuticle itself [CM mass per unit area is 1.0 to 1.3 $g \cdot m^{-2}$ (Knoche et al. 2004)] had no significant effect on water vapor permeance. It should be noted that these results were obtained using a "cast" film of uniform thickness within the cross-sectional area of water vapor transport. In the orchard, spray deposits would be more localized, so some hypothetical decrease in permeance would relate only to the small fraction of the fruit surface in contact with a spray deposit. For the solution properties of a Parka® application, this fraction averaged only about 22% of the whole fruit surface.

Table 6. Effect of Parka® on cracking of 'Sweetheart' sweet cherries after simulated rain.

| | Cracked fruit (%) | | |
|---|---|---|---|
| Treatment | 2021 | 2022 | Mean |
| Control | 25.5 ± 6.8 b[i] | 40.9 ± 7.9 a | 33.2 ± 10.5 a |
| 2 × | 27.1 ± 5.7 b | 57.3 ± 7.1 a | 42.2 ± 9.1 a |
| 12 × | 57.0 ± 2.9 a | 37.7 ± 7.4 a | 47.4 ± 7.9 a |

[i]Means within columns followed by the same letter are not significantly different (Tukey's Studentized range test, $P \leq 0.05$).

Fruit were sprayed with Parka® twice (after petal fall and at straw yellow, 2 ×) or 12 times (weekly, 12×) starting at petal fall. Untreated fruit served as control. Artificial rain was simulated in a fog chamber using deionized water. The percentage of cracked fruit on each tree was quantified. Data represent means ± standard errors.

## Conclusions

Rain cracking can often be economically disastrous in rain-susceptible fruit crops, such as sweet cherry. There are many claims that proprietary spray-on product can mitigate this disorder. One such product is Parka®, which claims to reduce damage from moderate rainfall before harvest in cherry. It does this by fortifying the cuticle by supplying a cuticle precursor. However, the manufacturers indicate their product should be applied late. This timing turns out to be after cuticle deposition has ceased, following downregulation of the cuticle synthesis genes. Our results fail to discover any evidence that Parka® is or might be effective. Furthermore, film formers with a contact mode of action that supposedly reduce the permeance of the fruit skin to water bear the risk of also reducing the permeance of leaf surfaces to gas exchange in photosynthesis. These are inevitably two sides of the same coin. Although rain shelters are both capital and labor intensive and may fail under severe conditions, they remain the only effective way of mitigating rain cracking in sweet cherry.

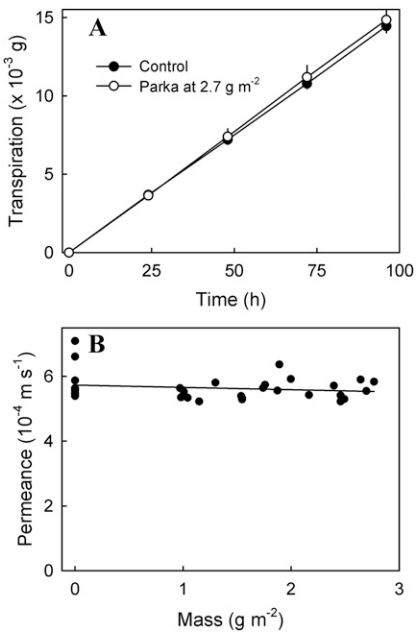


Fig. 4. Effect of Parka® on the time course of transpiration through coated polycarbonate sheets (**A**) and on the water vapor permeance of coated polycarbonate sheets (**B**). Parka® films were produced by casting solutions on polycarbonate sheets. The thickness of the Parka® film is indexed by the mass per unit area of the film. Uncoated polycarbonate sheets served as control. For comparison, the permeance of an uncoated sweet cherry fruit skin averages $1.15 \times 10^{-4}$ $m \cdot s^{-1}$ (Knoche et al. 2000).

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