Characterization of NO2 Bound to the Plant Cuticle by FT-IR Spectroscopy

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Nitrogen oxide binding to isolated tomato fruit cuticles has been characterized using Fourier transform infrared spectroscopy. The performed infrared analysis indicates that nitration occurs in the flavonoids of mature tomato cuticles.

The plant cuticle (cuticular membrane) is a continuous nonliving, lipophilic material that forms the interface between the plant and the environment [1]. The cuticle plays an important role controlling the penetration of herbicides, plant growth regulators and hazardous chemicals [2]. In this sense, the cuticle serves as the prime barrier to the sorption and subsequent uptake of xenobiotics deposited from the atmosphere [3]. Nevertheless, checking and identifying exogenous chemicals at the cuticular level currently requires a combination of distinct destructive techniques including labelled compounds [4]. It seems of interest to search new experimental approaches to yield more information from the structural point of view and, in addition, with less time consuming analysis.

Recently, our research group reported the usefulness of Fourier transform infrared spectroscopy (FT-IR) for structural studies on isolated cuticles [5]. This communication reports the use of this approach to describe interactions between cuticular membranes and exogenously applied chemicals. For this purpose, the interaction of nitrogen oxide with isolated cuticles has been selected.

It has been documented that nitrogen oxide, an air pollutant from diverse combustion processes, interacts with the plant cuticle and that the exposure of isolated plant cuticles to NO_2 shows irreversible uptake of the pollutant [6, 7]. These facts give to the cuticle/nitroxide system a high ecotoxicological interest.

To perform our study, cuticles from mature tomato fruits (*Lycopersicon esculentum* Mill.) were isolated as described elsewhere [8]. Isolated cuticles kept at about 20 °C and a relative humidity at 60% were then exposed to 100% NO_2 in a small glass chamber. After 6 h the cuticles were removed from the chambers, stored during 1 h in air at 20 °C to desorb gaseous NO_2 and washed three times (15 min each) with desionized water (1 ml water/mg cuticle). Afterwards the cuticles were placed in a desiccator and were brought to equilibrium with air over dry silica gel at room temperature.

Infrared spectra were obtained with a Perkin-Elmer 1760X Fourier transform infrared spectrometer. The spectra were obtained on treated and untreated dry cuticular membranes as described by Ramírez *et al.* [5]. Spectra recorded from different samples of cuticular membranes gave essentially the same relative absorption bands.

Infrared spectrum between 1800 and 600 cm^{-1} of untreated and NO_2 exposed cuticles are shown in Fig. 1 and 2, respectively. The two spectra seem almost identical and only after a detailed study of the assignments one can elucidate the differences between the two isolated cuticles. While the FT-IR

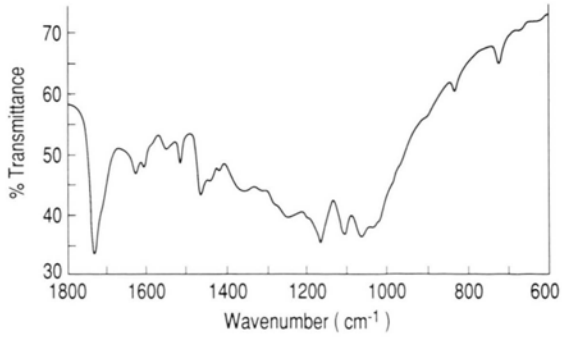

Fig. 1. Fourier transform IR spectrum of isolated tomato fruit cuticular membrane in the 1800–600 cm^{-1} region.

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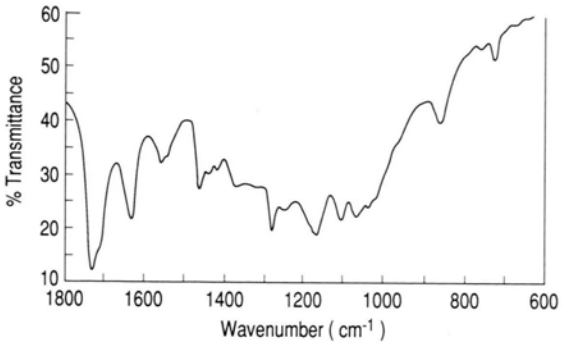

Fig. 2. Fourier transform IR spectrum of treated (NO_2 exposure) isolated fruit cuticular membrane in the 1800–600 cm^{-1} region.

spectrum of untreated tomato cuticle shows the absorption bands discussed by Ramirez *et al.* [5], the infrared spectrum of the treated cuticle yields new bands in addition to some shifted frequencies in comparison to those observed in the untreated cuticular membrane. The new absorption bands appear at 1631, 1278 and 860 cm^{-1} and they have been assigned to different nitrogen-oxygen vibrations [9]. The first two frequencies correspond to asymmetrical and symmetrical stretching vibrations of the NO_2 group, respectively. The symmetric stretching vibration (at 1278 cm^{-1}) appears in a spectral region where the untreated cuticle has no significant infrared absorptions. The asymmetric stretching can be observed as a broader band due to the chemical modifications that the bound nitrogen oxide induces in the tomato cuticle. The same fact was observed upon analyzing the infrared absorption found at 860 cm^{-1} in comparison with the absorptions that were measured in the untreated tomato cuticular membrane; this band has been assigned to the NO_2 bending vibration. A summary of these observed significant differences in the absorption frequencies and their assignments is given in Table I.

Taking into account the chemistry and composition of the plant cuticle, one may expect that phenolics are the most probable compounds for NO_2 binding. This hypothesis has been confirmed by Kisser-Priesak *et al.* [7] after selective extraction and GC analysis of depolymerized isolated tomato cuticles previously treated with nitrogen oxide.

Our findings indicate that there is effective NO_2 binding to selected phenolics present in the cuticular membrane and some observed spectral modifications seem to confirm this point. The noticeable

Table I. Significant frequencies (in cm^{-1}) measured in the IR spectrum of treated (NO_2 exposure) isolated tomato fruit cuticles.

Frequency	Intensity	Assignment[a]
1716	medium	ν (C=O)[b]
1631	strong	ν_a (NO_2)
1278	medium	ν_s (NO_2)
860	medium	δ (NO_2)

[a] ν = stretching vibration; δ = bending vibration a = asymmetric; s = symmetric.
[b] Carbonyl groups belonging to flavanones; this band appears at 1624 cm^{-1} in the spectra of untreated cuticles.

spectral change observed around 1620 cm^{-1} (see Fig. 1 and 2) can be interpreted in terms of different chemical arrangements of concrete phenolics with functional groups which absorb at these frequencies after nitrogen oxide treatment. Recent research has demonstrated that the infrared absorptions observed at 1624 and 1606 cm^{-1} in isolated mature tomato cuticles can be assigned to the β-hydroxy-ketone group present in the flavanone naringenin (Heredia *et al.*, unpublished data). After nitrogen oxide treatment the keto group shifts to higher frequency (1716 cm^{-1}, see Table I and Fig. 1 and 2) and only the NO_2 asymmetrical stretching vibration appears in the above mentioned spectral region. Thus, the absorption band located at 1730 cm^{-1} with a shoulder at 1713 cm^{-1} observed in the untreated cuticles and assigned to carbon oxygen stretching vibrations of the carbonyl group of ester bond [5] increases as a result of the above mentioned spectral shift. Additionally, small spectral changes observed around 1550 cm^{-1} agree with these structural considerations. Since these bands have been assigned to different aromatic stretching vibrations, nitration of aromatic rings modifies both the intensity and location of these absorption bands [5, 9].

It is known that the amount of naringenin and its chalcone derivative, chalconaringenin, present in mature tomato cuticles can reach five per cent of the cuticle weight [10]. These flavonoids are randomly distributed, trapped or covalently bound, in the polymer matrix of the cuticular membrane. Thus, the selective binding of nitrogen oxide to these cuticular components affects the major part of the tomato cuticle.

Summarizing, these results demonstrate the usefulness of infrared spectroscopy as a tool of *in situ*

identification of chemicals at the cuticular level. That is of great ecotoxicological importance in the case of irreversible uptake of herbicides or pollutants. In addition, the infrared spectroscopy can yield structural information on the special arrangement of the different functional groups present in this biopolymer.

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