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### Citation for published version:

McLeod, A, Fry, SC, Loake, GJ, Messenger, DJ, Reay, D, Smith, KA & Yun, BW 2008, 'Ultraviolet radiation drives methane emissions from terrestrial plant pectins', *New Phytologist*, vol. 180, no. 1, pp. 124-132.  
<https://doi.org/10.1111/j.1469-8137.2008.02571.x>

### Digital Object Identifier (DOI):

[10.1111/j.1469-8137.2008.02571.x](https://doi.org/10.1111/j.1469-8137.2008.02571.x)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Peer reviewed version

### Published In:

New Phytologist

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This is the author's final draft as submitted for publication. The final version was published in *New Phytologist* by Wiley Blackwell (2008) and is available online.

Cite As: McLeod, A, Fry, SC, Loake, GJ, Messenger, DJ, Reay, D & Yun, BW 2008, 'Ultraviolet radiation drives methane emissions from terrestrial plant pectins'*New Phytologist*, vol 180, no. 1, pp. 124-132.

DOI: 10.1111/j.1469-8137.2008.02571.x

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## **Ultraviolet radiation drives methane emissions from terrestrial plant pectins**

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## Summary

- Recent studies demonstrating an *in situ* formation of methane (CH<sub>4</sub>) within foliage and separate observations that soil-derived CH<sub>4</sub> can be released from the stems of trees have continued the debate about the role of vegetation in CH<sub>4</sub> emissions to the atmosphere. Here we report a study of the role of ultraviolet (UV) radiation in the formation of CH<sub>4</sub> and other trace gases from plant pectins *in vitro* and from leaves of tobacco *in planta*.
- Plant pectins were investigated for CH<sub>4</sub> production under UV irradiation before and after de-methylesterification and with and without the singlet oxygen scavenger 1,4-diazabicyclo[2.2.2]octane (DABCO). Leaves of tobacco were also investigated under UV irradiation and following leaf infiltration with the singlet oxygen generator rose bengal or the bacterial pathogen *Pseudomonas syringae*.
- Results demonstrated production of CH<sub>4</sub>, ethane and ethylene from pectins and from tobacco leaves following all treatments, that methyl-ester groups of pectin are a source of CH<sub>4</sub>, and that reactive oxygen species (ROS) arising from environmental stresses have a potential role in mechanisms of CH<sub>4</sub> formation.
- Rates of CH<sub>4</sub> production were lower than those previously reported for intact plants in sunlight but the results clearly show that foliage can emit CH<sub>4</sub> under aerobic conditions.

**Key words:** methane, pectin, vegetation, reactive oxygen species, *Nicotiana tabacum* (tobacco), ultraviolet (UV) radiation, DABCO.

## Introduction

There remains considerable discussion among plant physiologists about recent observations of the production of CH<sub>4</sub> by vegetation foliage under aerobic conditions (Dueck & van der Werf, 2008). Observations by Keppler *et al.* (2006) first demonstrated that dry and live leaf material could produce CH<sub>4</sub>, with rates influenced by sunlight and temperature, and this was suggested as a possible explanation for unexpectedly high atmospheric concentrations of CH<sub>4</sub> detected over tropical rainforests (Bergamaschi *et al.*, 2007). Biospheric CH<sub>4</sub> emissions to the atmosphere are thought to originate mostly from anaerobic microbial processes but experimental observations (Keppler *et al.*, 2006; Sanhueza & Donoso, 2006; Wang *et al.*, 2008), atmospheric measurements (Crutzen *et al.*, 2006; do Carmo *et al.*, 2006; Miller *et al.*, 2007) and subsequent analyses (Bousquet *et al.*, 2006; Houweling *et al.*, 2006; Ferretti *et al.*, 2007) do suggest the possibility of a modest CH<sub>4</sub> flux from terrestrial vegetation. Two subsequent investigations were unable to demonstrate CH<sub>4</sub> emissions (Beerling *et al.*, 2007; Dueck *et al.*, 2007) but a recent study, using measurements in the dark, has suggested that some woody plants, but not grasses, may emit small amounts of CH<sub>4</sub> (Wang *et al.*, 2008). The CH<sub>4</sub> was hypothesised to originate from methoxyl groups (Keppler *et al.*, 2006), and recently Keppler *et al.* (2008) have used isotope analysis to demonstrate an influence of temperature and UV radiation on CH<sub>4</sub> emissions from pectin and polygalacturonic acid *in vitro*. The roles of UV radiation and temperature in CH<sub>4</sub> emission from leaves have also been demonstrated by Vigano *et al.* (2008) while observations that CH<sub>4</sub> emissions from vegetation may arise from dissolution of soil CH<sub>4</sub> and its transport above ground in the transpiration stream (Rusch & Rennenberg, 1998; Terazawa *et al.*, 2007) has widened the debate about possible CH<sub>4</sub> sources in vegetation. At present, the mechanisms by which terrestrial

vegetation may emit CH<sub>4</sub> from foliage under aerobic conditions remain to be identified (Schiermeier, 2006, 2007; Dueck & van der Werf, 2008).

The role of temperature and sunlight in the observations of Keppler *et al.* (2006) and the well-known role of UV radiation in photodegradation of plant material (Austin & Vivanco, 2006) led us to investigate the role of UV radiation in CH<sub>4</sub> production from foliage. In earlier experiments, we found that leaf litter from oak trees grown under elevated UV-radiation had an accelerated decomposition rate and reduced extractability of carbohydrates that implied changes to cell wall components (McLeod *et al.*, 2007). Over half of plant primary cell wall dry mass may be comprised of pectins, which are cell-wall polysaccharides rich in  $\alpha$ -D-galacturonate residues with variable proportions of methylesterification (Seymour & Knox, 2002). We therefore investigated the release of CH<sub>4</sub> and other gases from pectin-impregnated glass fibre sheets, dried and exposed to UV radiation (from experimental lamps and from sunlight) inside gas-tight UV-transmitting bags filled with ambient air at 30°C. Care was taken in the experimental design to eliminate possible confounding effects from any contamination of the glass fibre or the release of low molecular weight hydrocarbons from the gas bag material. We also examined trace gas production from UV irradiated leaves of the C3 sub-tropical herb *Nicotiana tabacum* L. that were detached in order to limit transpiration as a possible CH<sub>4</sub> source. Sharpatyi (2007) recently suggested that a free-radical process would produce CH<sub>4</sub> from plant polysaccharides under the influence of UV-radiation, which is well-known to produce reactive oxygen species (ROS) in plant tissue (Björn, 2002). We therefore investigated CH<sub>4</sub> production from glass fibre impregnated with pectin and the ROS-scavenger DABCO (Wang *et al.*, 2006) and also from de-methylesterified pectin.

ROS are generated in foliage by a range of environmental stresses including: drought, nutrient deficiency, attempted pathogen infection, high temperature, acidic precipitation and ozone exposure (Apel & Hirt, 2004; Wang *et al.*, 2006). We therefore also compared the effects of UV irradiation on CH<sub>4</sub> production from tobacco leaves with those from leaves infiltrated with water, the singlet oxygen generator rose bengal (Filkowski *et al.*, 2004) or the bacterial pathogen *Pseudomonas syringae* in order to demonstrate the potential role of ROS in CH<sub>4</sub> formation.

## **Materials and methods**

### ***Pectin sheets***

Pectin or pectate-impregnated sheets (20.3 x 25.4 cm) were prepared from glass microfibre filters (Whatman GF/A) and commercial pectin derived from citrus fruits (Sigma Chemical Co.). Before use, glass fibre sheets were baked overnight in a furnace at 300°C in order to remove any organic contaminants. Citrus pectin (10 g; Sigma P9135; galacturonic acid content 84%; methoxy content 9.4%; loss on drying 4.1%) was wetted with 20 ml ethanol to form a slurry, which was dispersed in ~950ml deionised water with vigorous shaking. After several hours of stirring until dissolved to form a slightly hazy solution, the volume was adjusted to 1 litre with water. Pectate (de-methylesterified pectin) was prepared by addition of 400 ml of 1% pectin solution to 40 ml of 1.0 M NaOH, and incubation at 20°C for 30 min. Then, with vigorous shaking, sufficient 1 M H<sub>3</sub>PO<sub>4</sub> was added to bring the pH to 7.4–7.6. 25 ml of the pectin or pectate solution was then applied to each 20.3 × 25.4 cm sheet of Whatman GF/A glass microfibre filter and allowed to dry in air. With pectin, this resulted in 240 mg of polysaccharide (210 mg galacturonic acid residues) per sheet (equivalent to 23.5 mg (=760 μmol) methyl-ester groups per sheet, giving a theoretical maximum

yield of 12.1 mg CH<sub>4</sub> (~17 ml at STP)). With pectate, there was 175 mg galacturonic acid residue per sheet. Control sheets of glass fibre were prepared in the same way with 25 ml 2% ethanol. Pectin sheets containing DABCO (1,4-diazabicyclo[2.2.2]octane) (Sigma Chemical Co.) were prepared as above except that the pectin solution contained 8 mM DABCO. The absorbance spectrum of a 2.5 g l<sup>-1</sup> solution of pectin in distilled water was measured in a scanning spectrometer (Perkin Elmer Lambda 900 UV/VIS/NIR) for evaluation of the UV absorbance of pectin.

### ***Plant growth and leaf infiltration***

Tobacco plants (*Nicotiana tabacum* L. cv. Xanthi) were grown from seed in a temperature regulated glasshouse using 7.5 l pots containing a mixture of 75% peat and 25% sand with 750g ground limestone, 1200g ‘Osmocote Exact High K’ slow release fertilizer (NPK 10:11:18 + 2 MgO + trace elements) (The Scotts Company) and 6 g ‘Intercept 60WP’ insecticide (a.i. imidacloprid) (Bayer Environmental Science) per 250 l soil mixture. Day/night temperature was 21/18 °C, light intensity ~150 μmol m<sup>-2</sup> s<sup>-1</sup> and daylength extended to 18 h with supplemental sodium lighting. After 8-10 weeks, the youngest fully-expanded leaves were infiltrated on the abaxial surface with a 1 ml syringe with 2 ml of either distilled water, 10 μM rose bengal in water (Sigma Chemical Co.) or a water suspension (0.2 OD<sub>600</sub>) of *PstDC3000(avrB)*. The bacterium was grown in King’s broth (KB) liquid medium (King *et al.*, 1954) supplemented with 50mg l<sup>-1</sup> rifampicin and 50mg l<sup>-1</sup> kanamycin at 30°C overnight. Bacterial cells were harvested by centrifugation and re-suspended to 0.2 at OD<sub>600</sub> (the equivalent of 10<sup>8</sup> cfu ml<sup>-1</sup>) in 10mM MgCl<sub>2</sub>.

### ***Ultraviolet radiation sources***

UV radiation was provided by three types of lamp (UV313, UV340, UB351, The Q-Panel Company) filtered with closely-wrapped 125  $\mu\text{m}$  cellulose diacetate, which had a shortwave cutoff at approximately 290 nm (CA lamp filter, Fig. 1b) and so removed ultraviolet-C wavelengths ( $<280\text{nm}$ ). We also used a filter of 0.036 mm UV-opaque polyester (CG lamp filter) ('Courtgard', CPFilms Inc.) to remove UV-B and most UV-A wavelengths ( $<380\text{ nm}$ ). Examples of the spectral irradiance of lamp/filter combinations, including the gas sampling bag, are shown in Fig. 1b. Lamp irradiation was adjusted using a phase-angle dimming system. Experiments performed in natural sunlight took place in the horticultural gardens of the University of Edinburgh at UK National Grid Reference NT 270705 ( $55^{\circ} 55' \text{ N}$ ,  $3^{\circ} 10' \text{ W}$ ), between 6 and 21 September 2006.

### ***Ultraviolet radiation measurements***

Ultraviolet spectral irradiance was measured with a double monochromator spectroradiometer (SR991-PC, Macam Photometrics) which was calibrated against tungsten and deuterium lamps traceable to National Physical Laboratory Standards (SR903, Macam Photometrics). During outdoor experiments the solar spectrum was scanned at approximately 15 min intervals and monitored continuously with a broad band UV sensor (Model PMA2102, Solar Light Inc.) that was used to calculate changes in spectral irradiance between scans.

### ***Preparation of sample bags***

New 5 l gas sampling bags of 25  $\mu\text{m}$  UV-transparent polyvinylfluoride film (SKC Inc.) were cut open on one side to insert glass fibre sheets and re-sealed using 40  $\mu\text{m}$



Al adhesive tape. Bags were flushed five times before filling with 250 ml of stock external ambient air. Each pair of sample bags was used for three replicate experiments (which were determined not to have modified UV transmission of the bag material). Experiments using rose bengal, *P. syringae* and UV with tobacco were performed using 200 ml air and a 20 cm square window of 4 mm ‘Sanalux’ glass (Deutsche Spezialglas AG, Delligsen, Germany) attached with Al tape and the remainder of the bag shaded with Al foil. The spectral transmissions of polyvinylfluoride film, Sanalux glass and a range of typical chamber and cuvette construction materials were measured using a scanning spectrometer (Perkin Elmer Lambda 900 UV/VIS/NIR) and are shown in Fig. 1c.

### ***Experimental exposures of pectin and leaves***

Experiments on pectin with UV lamps used one sample bag containing one pectin-impregnated glass fibre sheet and another sample bag containing a control glass fibre sheet. Bags were supported on a black butyl rubber sheet (pond liner) on the surface of a thermostatically controlled water bath at 30°C. After two hours, the CH<sub>4</sub> production was determined and then the pectin-impregnated and control sheets were reversed between bags for a further 2-hour exposure. This allowed any difference in CH<sub>4</sub> production from the sample bags themselves to be eliminated from the estimate of CH<sub>4</sub> production from pectins. The CH<sub>4</sub> production inside control bags containing control glass fibre sheets was up to 6.6 ng CH<sub>4</sub> h<sup>-1</sup> in sunlight and a maximum of 24.6 ng CH<sub>4</sub> h<sup>-1</sup> in lamp experiments at the highest irradiance of UV313 lamps. Outdoor experiments were performed with bags clipped to a temperature-controlled brass plate also covered with black butyl rubber sheet. Temperature was measured inside a sample bag with thermocouples connected to a PC-based control system that adjusted

the temperature of water from a re-circulating water bath. As outdoor UV levels were variable, experiments were conducted for one period of 2 hours without reversing the treatment and control bags. However, the bags were reversed before the next experiment.

Experiments with leaves of *N. tabacum* used one leaf per gas sample bag and were performed inside a growth room at 25-30°C with 18 W m<sup>-2</sup> photosynthetically-active radiation (PAR: 400-700 nm) provided by fluorescent lamps (Philips Master TLD 36W/830) and 3.1 W m<sup>-2</sup> unweighted total UV provided by CA-filtered UV313 lamps (described above). Experiments with PAR and with PAR plus UV radiation were also performed using empty bags as a control and the control gas production was subtracted from treatment values.

### ***Gas concentration measurement***

Methane, ethane and ethylene concentrations were determined with a gas chromatograph (Hewlett Packard Series II 5890) equipped with a flame ionisation detector and a column packed with HayeSep Q (80-100 mesh) at 70 °C, with N<sub>2</sub> as carrier gas. Peak integration and autosampling were controlled using a chromatography data system (PeakSimple Model 203). CO<sub>2</sub> was measured with a gas chromatograph (Perkin Elmer 8310) equipped with a thermal conductivity detector, using manual injection. Before and after sample analysis, the gas chromatographs were calibrated using standards of known mixing ratios that spanned the sample values.

### ***Statistical Analysis***

Rates of CH<sub>4</sub> production from pectin are reported as the mean of three replicates  $\pm$  SE and were analysed by linear regression against UV irradiance values. Trace gas concentrations produced by UV, *P. syringae* and rose bengal treatments of tobacco are also means of three replicates  $\pm$  SE and were analysed by individual treatment comparison with the water control using ANOVA with replicate gas bags nested within treatment to achieve a repeated measures analysis (Neter *et al.*, 1996). All statistical tests were performed with the MiniTab statistical package (Version 14.1).

### **Results and Discussion**

The experiments on pectin used fluorescent lamps (filtered to exclude wavelengths <290 nm) in order to provide both UV-B (280-315 nm) and UV-A (315-400 nm) radiation (Fig. 1b) at irradiance levels up to the highest global erythemal UV irradiance (Liley & McKenzie, 2006) (Table 1) and experiments were also conducted in the field with sunlight (Fig. 1b). The absorbance of a 2.5 g l<sup>-1</sup> solution of pectin (Fig 1c) demonstrated the presence of UV-absorbing components. Methane production from pectin (Table 1) had an approximately linear relationship to total UV irradiance for each lamp type alone or for sunlight, but considerable scatter when all sources were plotted together. We therefore calculated UV irradiance using a range of idealized (Micheletti *et al.*, 2003) and common spectral weighting functions (Table 1). Straight-line logarithmic weighting functions (e.g. Micheletti *et al.*, 2003) provided significant linear relationships with CH<sub>4</sub> emissions. The best fit was achieved by an idealized function that decayed one decade in 80 nm wavelength (Fig. 1a, inset) giving a significant linear regression between weighted-UV and CH<sub>4</sub> production (Fig. 1a).

These rates of CH<sub>4</sub> production exceed values previously reported for pectin with CH<sub>4</sub>-free air inside glass vials (Keppler *et al.*, 2006). Methane production in the dark was undetectable and was reduced to low levels by a UV-opaque filter (Table 1). De-methylesterification of the pectin also reduced CH<sub>4</sub> production to low levels (Fig. 1a).

As free-radical mechanisms could also produce carboxylate radicals and potential dimerisation of methyl radicals, we also examined the production of ethane, ethylene and CO<sub>2</sub>, as well as CH<sub>4</sub>, from UV irradiation of pectin and found production of all four gases with a molecular ratio of CH<sub>4</sub> : C<sub>2</sub>H<sub>4</sub> : C<sub>2</sub>H<sub>6</sub> : CO<sub>2</sub> of 1.0 : 0.1 : 0.2 : 240 at a total unweighted UV irradiance of 9.48 W m<sup>-2</sup> (Table 2). UV-induced production of ROS (Björn, 2002) is also a potential free-radical mechanism leading to CH<sub>4</sub> production from the methyl groups of pectins. We therefore examined CH<sub>4</sub> production from pectin-impregnated glass fibre containing the ROS-scavenger DABCO (Wang *et al.*, 2006) and found CH<sub>4</sub> production reduced to low levels (Fig. 1a) thus demonstrating a role of ROS in CH<sub>4</sub> generation from pectins. ROS include hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), the superoxide ion (O<sub>2</sub><sup>•-</sup>) and its non-ionised equivalent the hydroperoxyl radical (HO<sub>2</sub><sup>•</sup>), the hydroxyl radical (<sup>•</sup>OH), and singlet oxygen (<sup>1</sup>O<sub>2</sub>). Of these five, only <sup>•</sup>OH has been reported to cause extensive oxidative scission of plant polysaccharides (Fry, 1998; Fry *et al.*, 2001). However, DABCO is usually reported as a singlet oxygen scavenger (Wang *et al.*, 2006) thus implicating singlet oxygen in CH<sub>4</sub> generation. More detailed studies using a range of ROS-scavengers are needed to evaluate the precise molecular mechanisms of UV-induced CH<sub>4</sub> formation in this study and those of Keppler *et al.* (2008) and Viganò *et al.* (2008), which reported some CH<sub>4</sub> production from non-methylesterified organic material.

UV-photosensitising compounds (e.g. furocoumarins) are abundant in some plants; therefore ROS formation may be much greater in the foliage of certain plant species than in extracted pectins, although cuticular reflectance of UV and UV-screening compounds (McLeod & Newsham, 1997) will reduce the effective exposure of underlying structures. ROS are generated in foliage by a range of environmental stresses and we therefore also investigated the production of CH<sub>4</sub> from leaves of the C3 sub-tropical herb *Nicotiana tabacum* L. Infiltration of leaves of *N. tabacum* cv. Xanthi with the singlet oxygen generator rose bengal (Filkowski *et al.*, 2004) produced leaf necrosis while infiltration with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 carrying the avirulence gene *avrB* (*Pst*DC3000(*avrB*)) (Whalen *et al.*, 1991) resulted in a hypersensitive response (a genetically programmed cell death mechanism (Apel & Hirt, 2004)). All treatments resulted in some CH<sub>4</sub> formation but even greater amounts of ethylene and ethane over the subsequent 45 hours (Fig. 2). Ethane production was only detected after 5 h in all treatments. The amount of CH<sub>4</sub> production was significantly different from the water control for each of the three treatments (repeated measures ANOVA (UV:  $F_{1,4}=18.51$ ,  $P=0.013$ ; *P. syringae*:  $F_{1,4}=68.48$ ,  $P=0.001$ ; rose bengal:  $F_{1,4}=11.20$ ,  $P=0.029$ ) but the mean rate caused by UV irradiation was much greater than other treatments. Methane production caused by the UV treatment, which appeared linear with time over 45 h, was  $12.3 \pm 3.2$  ng g<sup>-1</sup> leaf dry weight h<sup>-1</sup>, which is similar to rates previously reported for detached leaves from a range of species (Keppler *et al.*, 2006) but much smaller than their reported rates for intact plants.

There is a potential for much higher UV-driven emissions at lower latitudes, where all sites between 50°S and 40°N experience peak erythemally-weighted (McKinlay & Diffey, 1987) UV irradiances that exceed  $0.25 \text{ W m}^{-2}$  (Liley & McKenzie, 2006). Sunlight-driven  $\text{CH}_4$  emissions from vegetation were suggested by Keppler *et al.* (2006) as a possible explanation for unexpectedly high atmospheric concentrations of  $\text{CH}_4$  detected over tropical regions by satellite remote sensing (Bergamaschi *et al.*, 2007; Schneising *et al.*, 2008). More recent studies have shown that the  $\text{CH}_4$  data retrieval was positively biased in tropical regions owing to spectroscopic interference by water vapour, but source inversions based upon an updated data retrieval method still point to substantial tropical  $\text{CH}_4$  emissions (Frankenberg *et al.*, 2008).

Converting low latitude erythemal irradiances  $>0.25 \text{ W m}^{-2}$  using typical spectra derived from a spectral radiation transfer model (Gueymard, 1995, 2001) and using the weighting function for  $\text{CH}_4$  production (Figure 1a inset) suggests equivalent values  $>11 \text{ W m}^{-2}$  on the  $\text{CH}_4$ -weighted irradiance scale of Fig. 1a and  $>30 \text{ W m}^{-2}$   $\text{CH}_4$ -weighted UV for the highest global irradiance measured at Cuzco, Peru (Liley & McKenzie, 2006). However, the relationship between appropriately-weighted UV radiation and  $\text{CH}_4$  production from plants *in vivo* (and also from dead plant material) should be determined over the full range of global exposure levels before up-scaling to estimates of global emissions as the relationship may not be linear at higher irradiance levels and may not persist through time as the substrate becomes modified.

The steep response of the process to shorter wavelengths (Fig. 1a inset) makes it essential to filter experimental lamps to remove wavelengths less than 290nm (McLeod, 1997) in order to ensure realistic exposures in experimental studies.

The use of detached tobacco leaves limited any potential contribution of CH<sub>4</sub> dissolved in the transpiration stream, but raised a question about the effect of leaf detachment on observed gas production. The leaves infiltrated with water served as a control and indicated negligible effect of leaf detachment on gas production, including stress ethylene, during the 45 h experiment. Ethylene is a well-known signal molecule in plants produced by environmental stress (Apel & Hirt, 2004; Wang *et al.*, 2006) and the effect of UV-B irradiation on ethylene production from aminocyclopropane-1-carboxylic acid in tobacco and consequent leaf damage has been reported by Nara & Takeuchi (2002). However, ethylene production directly from UV irradiation of pectins (Table 2) suggests a novel mechanism distinct from the classical ethylene biosynthesis pathways (Wang *et al.*, 2006) which may have implications for ethylene signalling.

These observations in which environmental stresses, particularly from UV irradiation, result in CH<sub>4</sub> emissions from foliage under aerobic conditions may contribute to resolving the mystery of CH<sub>4</sub> sources from terrestrial vegetation in daylight. However, the night time observations of CH<sub>4</sub> (Crutzen *et al.*, 2006) and experimental detection in darkness (Wang *et al.*, 2008) suggest that potential CH<sub>4</sub> sources from the transpiration stream (Terazawa *et al.*, 2007) should also be evaluated further. The rates of CH<sub>4</sub> production induced by rose bengal and *P. syringae* were much lower than those induced by UV irradiation, even though the general oxidative stress caused by these chemical and biological treatments are widespread in the leaf and caused cell death. The trace gas emissions caused by these treatments may originate from different processes (such as lipid oxidation) from those caused by UV irradiation. Nevertheless, the potential effects of ROS generated by other environmental stresses

should be considered. Experimental studies are generally undertaken using plants that have not been subjected to environmental stress or disease and without UV irradiation (e.g. Beerling *et al.*, 2007; Dueck *et al.*, 2007). Another likely reason why studies have failed to report a role of UV radiation in CH<sub>4</sub> production is because materials used in the construction of experimental leaf chambers and cuvettes, such as glass, polymethyl methacrylate (PMMA, 'Perspex' or 'Plexiglas') and polycarbonate do not transmit all UV-wavelengths (Fig. 1c), and plastic polymers may themselves release hydrocarbons under UV-irradiation (Lonneman *et al.*, 1981). The suggestion (Schiermeier, 2006) that some plant species emit up to 4000 times more CH<sub>4</sub> than others may reflect the variability of leaf structures, UV-screening pigments (McLeod & Newsham, 1997), UV-photosensitisers (Björn, 2002) and ROS-scavenging mechanisms (Apel & Hirt, 2004) found in plants. Previous measurements of CH<sub>4</sub> production from vegetation inside glass and 'Plexiglas' chambers (Keppler *et al.*, 2006; Dueck *et al.*, 2007) would have excluded some of the more energetic shorter wavelength UV in solar radiation so that rates of CH<sub>4</sub> emission in the field may be larger than suggested from past experiments. However, reported experimental CH<sub>4</sub> emissions (Keppler *et al.*, 2006; Sanhueza & Donoso, 2006) may also reflect a combination of experimental stress factors that could include heat, desiccation (and UV irradiance) as well as possible artefacts suggested by Kirschbaum *et al.* (2007). Sharpatyi (2007) also implied that gamma radiation is a driver of free radical mechanisms that may lead to CH<sub>4</sub> production and consequently the effect of gamma sterilization of vegetation samples to eliminate microbial effects (e.g. Keppler *et al.*, 2006) should also be tested carefully to ensure that it does not cause biochemical changes that influence subsequent experimental results.



## Conclusions

These results provide further evidence that plant pectins can act as a source of CH<sub>4</sub> under aerobic conditions when exposed to UV radiation within the ambient range (280-400nm) from experimental lamps and sunlight. They also provide a first step in understanding the potential mechanisms by demonstrating a role of ROS in CH<sub>4</sub> production and the additional production of ethane, ethylene and CO<sub>2</sub>.

The rate of CH<sub>4</sub> production ( $\sim 13 \text{ ng g}^{-1} \text{ h}^{-1}$ ) from tobacco leaves was similar to values reported for detached leaves by Keppler *et al.* (2006), but much lower than their values using intact plants. Nevertheless, vegetation stress factors, especially UV radiation, may have wider implications for biosphere–atmosphere interactions, as any ROS-forming mechanism may have the potential to produce not only CH<sub>4</sub> but also other atmospheric trace gases such as C<sub>2</sub> hydrocarbons and the methyl halides from terrestrial plant sources. A deeper understanding of the biochemical mechanisms for CH<sub>4</sub> production may provide the potential to minimise CH<sub>4</sub> generation from large-scale planting of crops and trees by selection for effective ROS-scavenging and/or UV screening properties. Consequently, the potential of high UV irradiance in the tropics and a range of environmental stress factors to cause ROS formation and trace gas emissions require examination in a range of species, using realistic spectral irradiance and experimental treatments, in order to fully understand the role of these processes in global emissions.

## **Acknowledgements**

We thank Julia Drewer, Caroline Nichol, Frank Keppler, Ann Webb, Nigel Paul, Ruth Doherty, Robert Vreeburg, Éva Hedig, Paul Palmer and Lesley Yellowlees for helpful discussions and Colin Kaye, Robert Howard, Alex Hart, Bill Adams, Sophie Haupt and Chris McLellan for assistance. This work was supported by research awards from the Natural Environment Research Council (to ARM), the Moray Endowment Fund (to ARM), the Biotechnology and Biological Sciences Research Council (to SCF, GJL and B-WY), a NERC Fellowship (to DSR), the University of Edinburgh Development Trust (to DJM) and the University of Edinburgh Donald Mackenzie Scholarship (to DJM).

**Table 1** Ultraviolet irradiance and CH<sub>4</sub> production from citrus pectin

Lamp source/location	Ultraviolet irradiance (W m <sup>-2</sup> )										Methane (ng g <sup>-1</sup> h <sup>-1</sup> )	
	Total UV (280-400 nm)	UV-A (315-400 nm)	UV-B (280-315 nm)	ERY <sup>a</sup>	PAS <sup>b</sup>	PGR <sup>c</sup>	QUT <sup>d</sup>	DNA <sup>e</sup>	STN <sup>f</sup>	MET <sup>h</sup>	Mean	s.e.
UV313-CA <sup>h</sup>	0.55	0.36	0.19	<0.01	0.09	0.08	0.12	0.07	0.23	0.30	53.1	4.0
	1.51	1.00	0.51	0.13	0.23	0.22	0.32	0.19	0.64	0.82	109.3	0.6
	2.07	1.37	0.70	0.18	0.32	0.31	0.44	0.26	0.87	1.13	201.2	11.4
	3.69	2.39	1.30	0.34	0.59	0.57	0.82	0.48	1.59	2.04	249.0	11.3
	5.70	3.61	2.09	0.55	0.95	0.92	1.31	0.79	2.52	3.20	361.9	8.2
	8.62	5.74	2.88	0.73	1.27	1.24	1.79	1.01	3.60	4.66	446.6	29.8
	9.48	5.55	3.93	1.10	1.90	1.82	2.53	1.66	4.52	5.62	660.8	36.7
UV340-CA <sup>h</sup>	3.70	3.56	0.14	0.02	0.03	0.10	0.10	0.02	0.60	1.07	143.0	3.8
	10.46	10.08	0.38	0.06	0.08	0.28	0.29	0.04	1.70	3.03	310.0	11.6
	20.35	19.49	0.86	0.13	0.19	0.58	0.64	0.10	3.51	6.13	644.6	29.8
UV351-CA <sup>h</sup>	5.27	5.21	0.06	0.01	<0.01	0.12	0.05	<0.01	0.61	1.26	146.3	6.6

	14.56	14.39	0.17	0.03	0.03	0.33	0.16	0.02	1.72	3.50	296.8	10.2
UV313-CG <sup>i</sup>	0.05	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.02	10.2	1.4
Dark	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-1.1	1.1
Sunlight	26.51	26.27	0.24	0.04	0.03	0.54	0.25	0.01	2.33	4.98	539.5	-
	7.98	7.88	0.10	0.02	0.01	0.17	0.11	<0.01	0.85	1.71	180.1	-
	22.60	22.40	0.20	0.03	0.03	0.46	0.21	0.01	1.98	4.25	498.3	-
	21.31	21.12	0.19	0.03	0.03	0.43	0.20	<0.01	1.87	4.01	448.5	-
	19.07	18.91	0.16	0.03	0.02	0.39	0.17	<0.01	1.67	3.58	391.4	-
Cuzco, Peru <sup>j</sup>	-	-	-	0.63	-	-	-	-	-	-	-	-

Values of CH<sub>4</sub> production are the mean of three replicate measurements for lamp sources and individual measurements in sunlight (see Methods).

<sup>a</sup> ERY: UV (280-400nm) weighted with the Commission Internationale de l'Eclairage (CIE) erythemal action spectrum (McKinlay & Diffey, 1987).

<sup>b</sup> PAS: UV (280-400nm) weighted with a mathematical formulation (Green *et al.*, 1974) of the general plant action spectrum (Caldwell, 1971).

- <sup>c</sup> PGR: UV (280-400nm) weighted with a new plant growth function (Flint & Caldwell, 2003).
- <sup>d</sup> QUT: UV (280-400nm) weighted with the pyridine dimer action spectrum (Quaite *et al.*, 1992).
- <sup>e</sup> DNA: UV (280-400nm) weighted with the DNA damage action spectrum (Setlow, 1974).
- <sup>f</sup> STN: UV (280-400nm) weighted with the plant growth inhibition function (Steinmuller, 1986).
- <sup>g</sup> MET: Idealized spectral weighting function for CH<sub>4</sub> production that decays one decade in 80 nm.
- <sup>h</sup> Lamps filtered with 125 μm cellulose diacetate which filters UV wavelengths <280 nm (see Fig 1b).
- <sup>i</sup> UV313 lamps filtered with 'Courtgard' polyester which filters UV wavelengths <380 nm (see Fig 1b).
- <sup>j</sup> Highest estimated global erythemal irradiation at Cuzco, Peru calculated from the UV index (Liley & McKenzie, 2006).

**Table 2** Production of trace gases from ultraviolet irradiation of citrus pectin

Methane (ng g <sup>-1</sup> h <sup>-1</sup> )		Ethane (ng g <sup>-1</sup> h <sup>-1</sup> )		Ethylene (ng g <sup>-1</sup> h <sup>-1</sup> )		CO <sub>2</sub> (μg g <sup>-1</sup> h <sup>-1</sup> )	
Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
660.8	36.7	125.2	14.1	271.0	9.5	433.4	3.6

Values are the mean of three replicate experiments. Total UV irradiance was 9.48 W m<sup>-2</sup> (280-400 nm) with corresponding weighted irradiance values using a range of common spectral weighting functions shown in Table 1.

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**Figure 1.** Methane production from citrus pectin. (a) Linear regression of CH<sub>4</sub> production with ultraviolet irradiance weighted with the inset spectral weighting function, using cellulose diacetate (CA) filtered lamps: UV313 (●), UV340 (◆), UV351 (◇), UV-opaque ‘Courtgard’ (CG) polyester-filtered UV313 lamps (★) and sunlight (▼). Open symbols correspond to spectra in Fig. 1b. For lamp sources, values are means of three replicates and standard errors are less than symbol size except where visible. For sunlight, values are individual measurements. Other symbols (close to the horizontal axis) show CH<sub>4</sub> production from pectin plus the ROS-scavenger DABCO (▲) and from de-methylesterified pectin (■). Temperature was 30 °C; (b) Spectra of sunlight (SUN) and CA-filtered lamps (UV313, UV340, UV351) and CG-filtered UV313 lamp (CG313) used in experiments; (c) Transmission spectra (solid lines) of filters used in experiments (CA, cellulose diacetate; CG, ‘Courtgard’ UV-opaque polyester) and of materials commonly used to construct experimental plant chambers (GL, 4 mm window glass; PE, 3.8 mm ‘Perspex’; PO, 2.7 mm polycarbonate, SA, 4mm ‘Sanalux’ glass). Absorbance spectrum (right-hand axis) of 2.5 g l<sup>-1</sup> pectin (PC) in water (hatched line).

**Figure 2.** Trace gas production from tobacco (*Nicotiana tabacum* var. Xanthi). (a) Methane; (b) Ethane; (c) Ethylene. Leaves were infiltrated with 2 ml of either water (○), a *PstDC3000(avrB)* suspension (□), a 10 μM rose bengal solution (●) or water plus UV irradiation (■). All treatments were under 18 W m<sup>-2</sup> (87 μmol m<sup>-2</sup> s<sup>-1</sup>) PAR (400-700 nm). The UV irradiation treatment was 3.14 W m<sup>-2</sup> (280-399 nm) (equivalent to 1.47 W m<sup>-2</sup> UV<sub>CH<sub>4</sub></sub> – see Fig. 1a) and temperature was 25-30 °C. Values are means of three replicates and standard errors are smaller than the symbol except where visible.

FIGURE 1

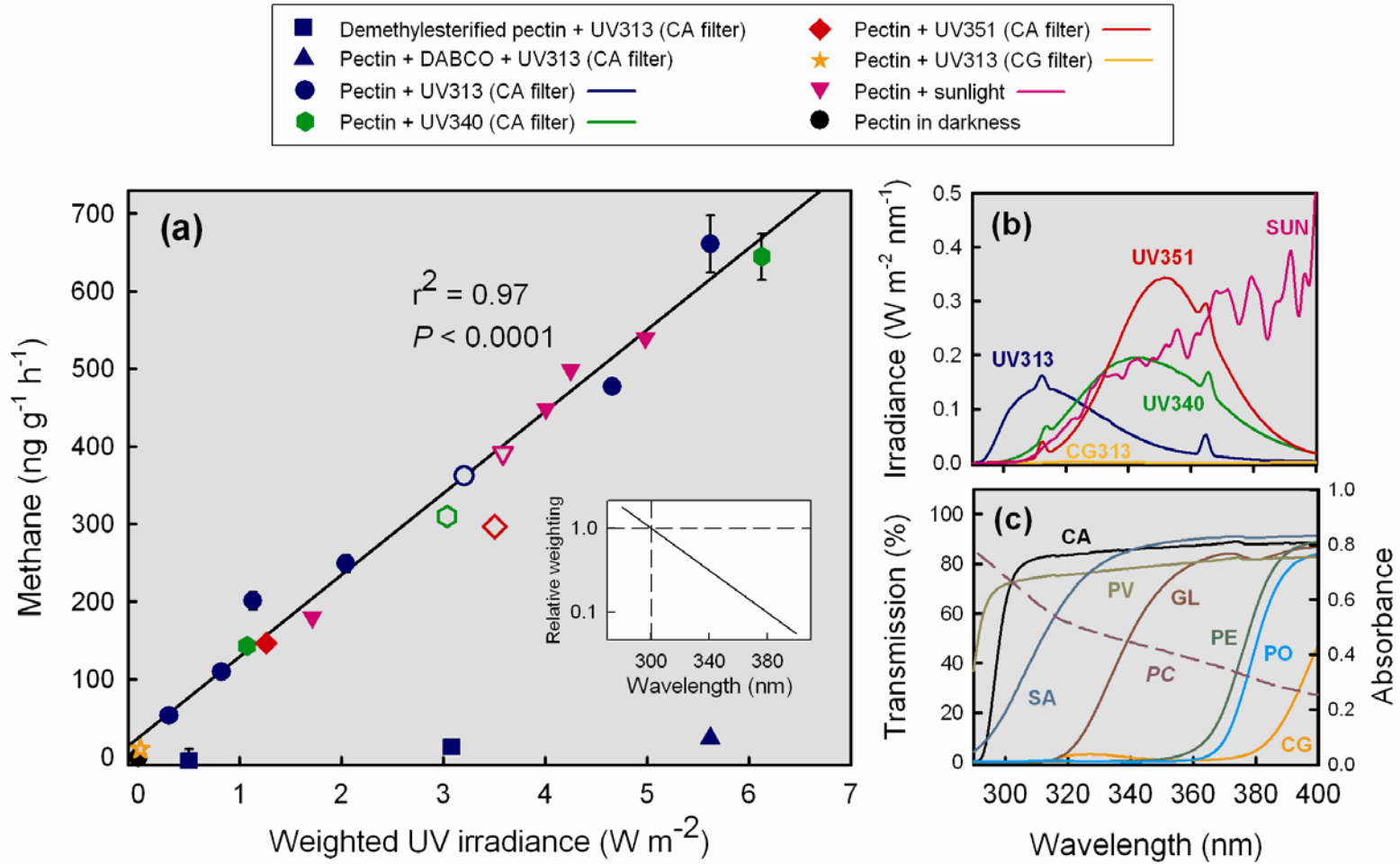


FIGURE 2

