Ethylene production in apple infected by *Gleosporium album* Ostrw. at cold storage

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ABSTRACT: In ten cultivars of apple fruit, ethylene production expressed in µl/kg/h was determined. The cultivar Resista exhibited a higher ethylene production and can be differentiated from other cultivars. The production ranged from 4.2 ± 0.58 µl/kg/h in the case of Meteor cv. up to 131.6 ± 5.5 µl/kg/h in Resista cv. Infected fruit of Topaz cv. had a lower ethylene production at cold storage temperature (3°C) than some healthy fruit. All examined cultivars can be divided into three clusters. Discriminant analysis and canonical correlation analysis of the examined apple fruit led to the determination of healthy and infected fruit. Values of ethylene production were analyzed on intact fruit by using headspace gas analysis by CGC with thermal desorption technique. Carbosieve G was chosen as the adsorbent material for the traps due to its relatively high affinity for light hydrocarbons such as ethylene. For a full trap of ethylene in the enrichment column the sufficient amount of percolating gas is about 0.3 l.

Keywords: *Gleosporium* rot; apple fruit; ethylene production; headspace gas analysis; cultivars

Ethylene is a gaseous plant hormone that, at very low concentrations, plays a major role in the regulation of the metabolism of harvested horticultural crops. The responses of harvested fruits, vegetables or flowers to endogenously produced and exogenously applied ethylene are numerous and varied, and they can be beneficial or detrimental depending on each case (Saltveit 1999). Both the synthesis and action of C_2H_4 involve complicated metabolic processes that require oxygen and are sensitive to elevated concentrations of carbon dioxide. Ethylene is responsible for an initiation of the softening of apple and other climacteric fruits (Liu 1977; Song, Bangerth 1996). Ethylene can influence the postharvest life of both climacteric and non-climacteric fruit by affecting their quality attributes and the development of physiological disorders and postharvest diseases (Kader 1985). A number of techniques to control the effects of ethylene are discussed in relation to their application to commercially important fruits. Lara and Vendrell (2003) suggested that increased endogenous ABA levels in the peel tissue might play a major role in cold-induced ethylene biosynthesis upon rewarming. Ethylene is also produced in copious amounts by diseased and injured tissue and mediates the defence responses of stressed tissue (Abeles et al. 1992).

Trace amounts of hydrocarbons as ethylene released from plant tissues into atmosphere are rarely in an amount sufficient for direct determination so preconcentration and FID/GC analysis are generally necessary. The concentration method for determining the ethylene transpired through the skin was provided by the work with impacted fruit. The analytes were taken off by the stream of percolation gas and were trapped in a concentrating column with porous polymer Porapak Q (Bečka, Feltl 1977) or Porapak P (Gelbičová-Růžičková et al. 1972) as sorbent. The concentrate obtained was analyzed by gas chromatography. The trapping of compounds to be examined can be carried out by both the conservation and the equilibration methods. It is necessary to consider analytical relationships for both the alternatives.

A method of the determination of ethylene produced by pulpy fruit or as a pollutant in the air was described (Goliáš, Novák 1985; Budrym, Hartman 1998) consisting in the equilibration trapping of ethylene in a column packed with Carbosieve G, the thermal desorption of deposit, and an analysis of the concentrate by gas chromatography (the flame ionization detector). Carbosieve SIII is used to adsorb ethylene from prepared standards and humid air. Even recent advances of the column technology have kept detection limits for ethylene in low ppm range without the use of expensive specialized detectors.

The marketing of organic farming products has markedly expanded because of an increased con-

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sumers' demand for healthy food products that are free of synthetic chemical residues with resulting improvements in the production and distribution systems (Sylvander 1993).

However, as organic fruits are not treated with chemical fungicides, they suffer from relatively high rates of decay that develops during the storage and shelf life. There has been an increasing interest in the use of pre-storage heat treatments to control the insect pests, to prevent the fungal decay and to modify the ripening of commodities (Lurie 1998). *Gleosporium* rot, the most dangerous post-harvest disease of organic apples can lead to over 50% loss during storage. The first appearance of *Gleosporium* disease can be observed after a few months of cold storage or at the latest when the apples are moved out of the storage room and in market. The reduction of *Gleosporium* rot under 10% after a storage time (five to six months) could be achieved with a hot water treatment (Trierweiler et al. 2003). Alternatives to chemical control, when used alone, are generally less effective than fungicides (Leverentz et al. 2000).

Too many bacteria and fungi have the ability to produce gaseous compounds belonging to ethylene and other gaseous metabolites. Qadir et al. (1997) show that *B. cinerea* demonstrates the capacity to produce ethylene in the presence of methionine. It is not known if the biosynthesis of ethylene in *B. cinerea* proceeds through the ACC (Yang, Hoffman 1984). The volatile profile of *M. albus*-colonized grain showed that 2-methyl-1-butanol and isobutyric acid were the major volatile compounds found in the headspace, which could be an attractive biological fumigant for controlling post-harvest diseases (Mercier, Jiménez 2004).

The objectives of this paper were to evaluate the influence of exposure to obvious ethylene concentration during a long-term storage at low temperature on the development of postharvest *Gleosporium* rot. On selected cultivars of apple fruit the development of postharvest *Gleosporium* rot was determined in relation to the ethylene production during cold storage. Effects of a cultivar of apple fruit on the ethylene production were also assessed.

MATERIAL AND METHODS

Fruit preparation

Ethylene production during cold storage and fruit quality were assessed in ten cultivars of apple fruit. Fruits for ethylene analysis were commercially grown; fruits for decay development assessment were purchased from a research planting, selected, randomized, and used after one month in the experiments.

Sampling fruit for ethylene measurement

An intact fruit was placed into a 500 ml sampling vessel that was permanently ventilated by dehumidified air with a flow rate of 50 ml/h. Ethylene released from samples into the percolating gas (air) was trapped on the sorbent in an enrichment column. However, the gas-solid system with Porapak Q was unsatisfactory for the determination of ethylene concentrations in percolating gas lower than 1 ppm. Therefore, gas-solid systems with the activated charcoals termed Carbosieve G and Carbosieve SIII as sampling tube packing were tried out. Carbosieve G was selected as adsorbent material for the traps for its relatively high affinity for light hydrocarbons and a better release of ethylene from this sorbent during the thermal desorption process. The weight of the analyzed fruit was about 180 g. The time of the percolating gas passing over the enrichment column was controlled of flow rate with ultimate volume 0.3 l.

Water vapour both from the air and from the sample was removed by drying column. Partial segments were in the following sequence: sampling vessel – drying tower – enrichment column – capillary flowmeter – needle valve – suction pump.

Specific retention volume of ethylene on sorbents Carbosieve G

The sorbents Carbosieve G is specially prepared activated charcoal with high specific inner surface about 1,000 m^2/g of adsorbents, which effectively provides the capacity to archive a concentration of ethylene. The value V_g was used to determine the relationship among the desorption temperature, gas volumes and desorption times required to thoroughly elute the analytes of the resin bed for quantitative analysis. Measurements were performed between the temperatures of 30 and 90°C. For the relationship between log V_g and the reciprocal value of absolute temperature (1/T) to be a true linear regression curve (with correlation coefficient $r = 0.99986$):

$$\log V_g = 1{,}720.430554/T - 2.499852 \quad (1)$$

Working method and calculation of results

The enrichment column containing the sorbent Carbosieve G of a weight of W_s 370 mg was inserted at a temperature of 20–25°C and flow rate 50 ml/min for a 6-minute flow through percolating gas, whereas total volume was 300 ml. Under these suction conditions ethylene was completely retained in the enrichment column (conservation version). Each sample

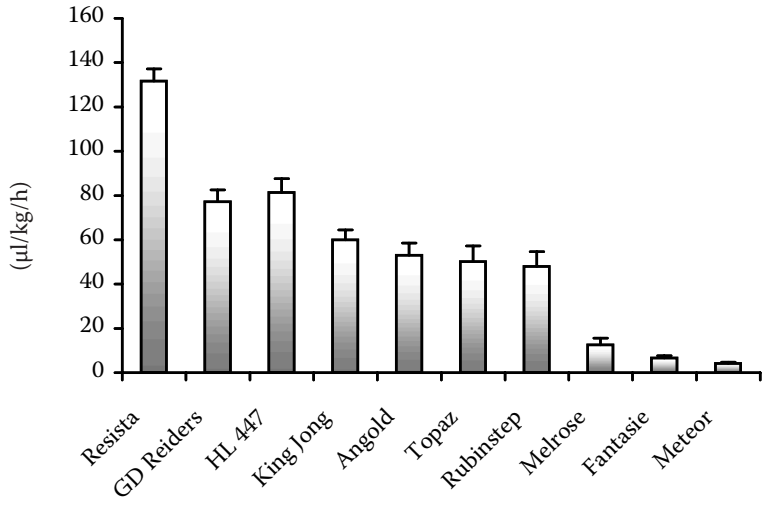

Fig. 1. Ethylene production of ten cultivars of apples. Vertical bars represent the standard error of the mean of ten replications, $P < 0.05$

was analyzed using a thermal desorption system model TD-2 operated in manual mode and an Agilent 4890D gas chromatograph with FID detection. Identities of ethylene and other compounds in the sample were confirmed in a separate analysis. The ethylene production from the impact fruit involved in percolating gas G_i is given by:

$$G_i = (A_i/A_s)v_{(s)}c_{(s)}F/G\,V \quad (2)$$

where: A_i – the peak surface in the chromatogram examined sample,
A_s – the peak surface in the chromatogram of ethylene in the calibration sample,
$v_{(s)}$ – the volume injected in the analytical column,
$c_{(s)}$ – the concentration of the standard in the calibration sample,
F – the flow rate of the percolating gas,
V – the volume permeated through the enrichment column,
G – the weight of the inserted fruit in the sampling chambers.

Control of *Gleosporium* rot of apple by artificial infection

The storage pathogen used in this study was isolated in the Research Institute of Pomology in Holovousy laboratory from infected plant material provided by Dr. Kloutvarová. Experiments were conducted with commercially grown apples and cultivars (Fig. 1) derived from the Research Institute of Pomology in Holovousy. Fruits were selected for the coloration of injuries and infections and placed in cold storage at 3°C. Each fruit was wounded on two locations at its equator with a 4 mm long metal nail tip. Then, 3 µl of conidial suspension of *G. album* (1×10^4/ml) were crushed into each wound. The control consisted of inoculated fruits in boxes with no colonized fruit. The boxes were kept at ambient air temperature (20–23°C) and the infection was measured before the ethylene analysis. Each experi-

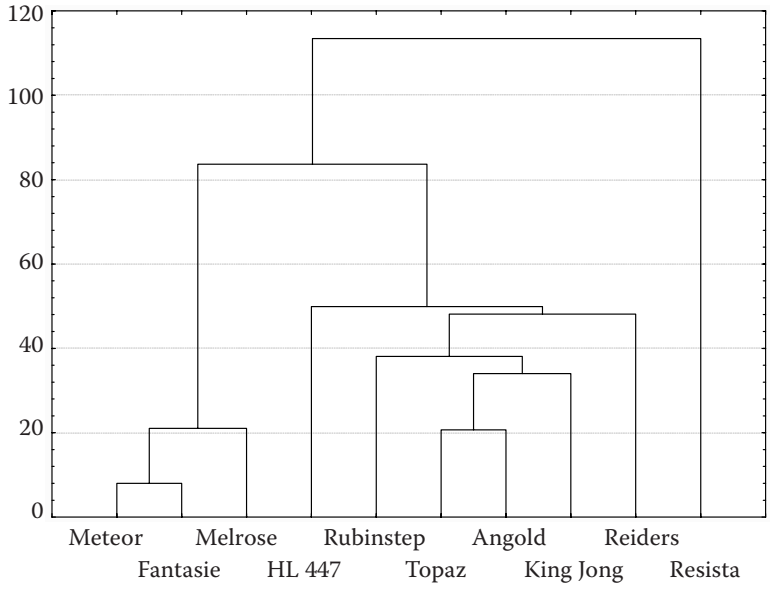

Fig. 2. Cluster analysis using the GC peak areas as the input data with a significance level of $P < 0.05$

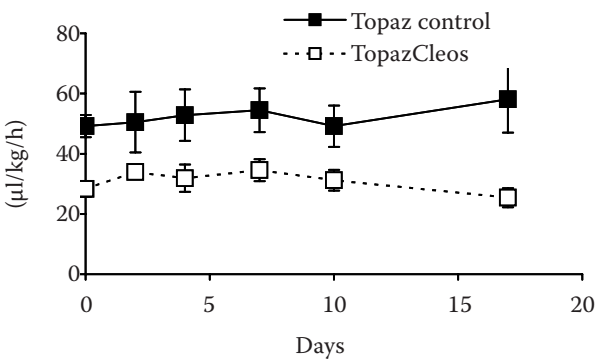

Fig. 3. Ethylene production of healthy fruit cv. Topaz (Topaz control) and fruit infected of *Gleosporium* rot. Vertical bars represent the standard error of the mean of ten replications, $P < 0.05$

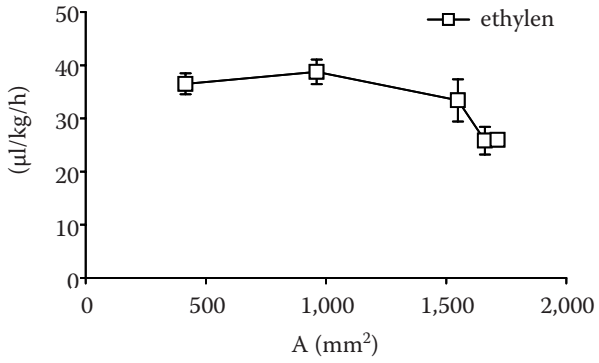

Fig. 4. Ethylene production versus infected area of *Gleosporium* rot. Vertical bars represent the standard error of the mean of ten replications, $P < 0.05$

ment was conducted in a completely randomized design with three replicate boxes with nine fruits each.

Decay development assessment

Brown rot incidence was determined weekly by counting the number of decayed fruit in each container. Brown rot severity was assessed in each decayed fruit as lesion diameter. To evaluate the number of infected fruit surrounding the central inoculated fruit it was counted weekly.

RESULTS AND DISCUSSION

Ethylene production of apple cultivars in cold storage

The introduction of new cultural practices, cultivars, harvest and handling methods, post-harvest treatments, consumer products and packaging influences the effect that C_2H_4 has on quality attributes. Once the ripening of climacteric fruit has started, the internal C_2H_4 concentration quickly increases to saturation levels and exogenous application of C_2H_4 has no further promotive effect on ripening. Cultivars in cold storage strongly differ in their ethylene production (Fig. 1). Higher ethylene production is exhibited in cultivar Resista, which could differentiate it from other cultivars. All examined cultivars can be divided into three clusters (Fig. 2). Genetic engineering may be able to dissect the biochemistry and physiology of ethylene and to produce fresh fruit with specifically designed responses to ethylene. For the current storage technology cultivars with lower ethylene production bring many advantages. In the case of low ethylene controlled atmosphere technology even a lower amount of ethylene should be removed from ambient atmosphere at storage by the ethylene scrubber equipment. Palou et al. (2003) observed no general commercial benefit that could be expected from actively removing ethylene from cold storage rooms containing stone fruit or table grapes.

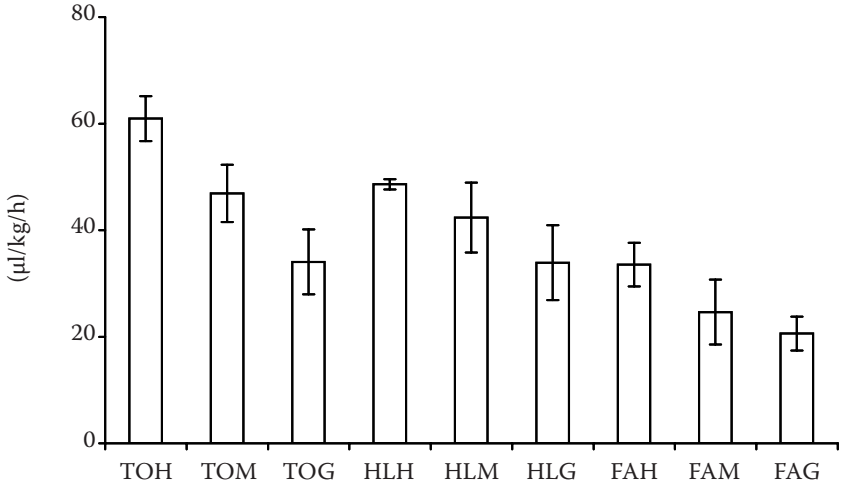

Fig. 5. Ethylene production of three cultivars of apple fruit (TO – Topaz, HL – cultivar HL 447, FA – cultivar Fantasie), common H – healthy, M – middle, G – *Gleosporium*, TOH – cultivar Topaz as healthy. Vertical bars represent the standard error of the mean of ten replications, $P < 0.05$

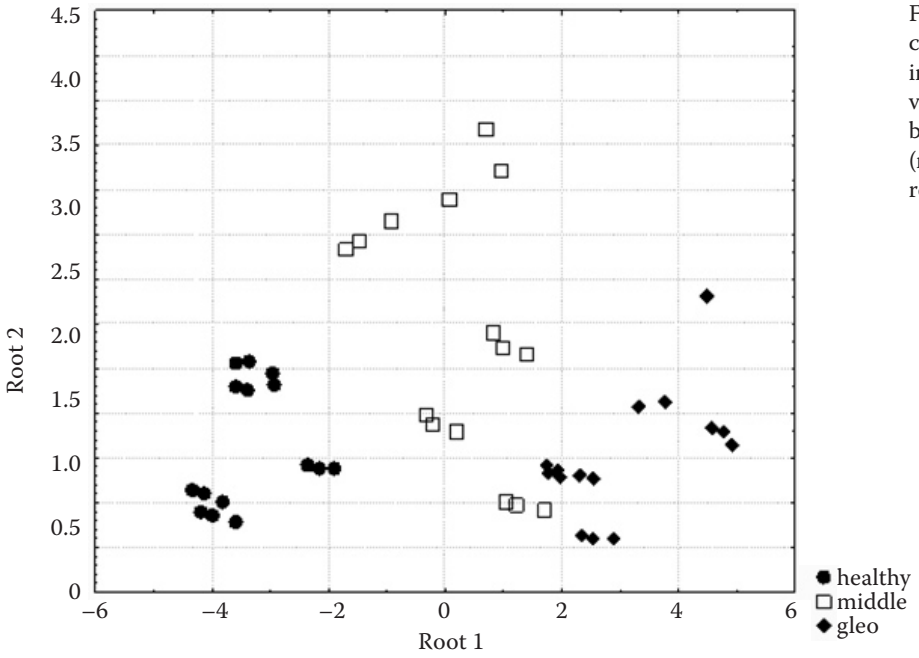

Fig. 6. Discriminant analysis and canonical correlation analysis with input data from 45 apple fruit divided into three groups (healthy), beginning of *Gleosporium* rot (middle) and apparent *Gleosporium* rot (gleo)

Ethylene production released from infected fruit

Our results showed that infected apples of cv. Topaz had lower ethylene production at the cold storage temperature 3°C than some healthy fruit (Fig. 3). Growth of the fungus was determined visually. Measurements were performed in the middle of storage time if the infected area was smaller than 40 mm^2 on fruit with weight about 200 g. When the area of the damaged surface increased, then the amount of released ethylene decreased (Fig. 4). Similar results were achieved in assessing apple fruits categorized into three groups: healthy, medium-damaged and highly infected areas (*Gleosporium* disease) (Fig. 5). Due to the diseased and injured tissue ethylene production was retarded. Another point of investigation in this study was the determination of ethylene production according to the degree of damage to the surface area. By the assessment of the great number of fruit, healthy and infected fruits can be well assorted using the discriminant analysis (Fig. 6). It is possible to assume that other non-ethylene volatile substances coming over into the ambient atmosphere through the diffusion process modified the authentic composition of the gas mixture. Investigation of the internal quality of apples treated with hot water showed no difference between the treated apples and the untreated control apples (Trierweiler et al. 2003). Overall, the rate of ethylene production of the fruit treated with hot water following cold storage was lower than that of the control fruit (Klein, Lurie 1990).

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Produkce etylenu v jablkách infikovaných *Gleosporium album* Ostrw. během chladírenského skladování

ABSTRAKT: V deseti odrůdách jablek byla stanovena produkce etylenu vyjádřená v µl/kg/h. Nejvyšší produkci etylenu vykazovala odrůda Resista, která se výrazně odlišovala od ostatních odrůd. Produkce etylenu odrůdy Meteor byla 4,2 ± 0,58 µl/kg/h, u odrůdy Resista byla 131,6 ± 5,5 µl/kg/h. Infikované plody odrůdy Topaz měly nižší produkci etylenu než plody zdravé. Všechny odrůdy je možné rozlišit do tří klastrů. Pomocí diskriminační analýzy s kanonickou korelační analýzou bylo dosaženo rozlišení plodů zdravých a plodů infikovaných. Hodnoty produkce etylenu byly získány z neporušených plodů, z nichž se etylen zachytil do sorbetu metodou headspace gas analýzy. Sorbent Carbosieve G byl vybrán pro zachycení kvůli své relativně vysoké afinitě pro lehké uhlovodíky, k nimž patří etylen. Pro úplné zachycení etylenu do obohacovací kolony dostačuje prosát 0,3 l perkolačního plynu.

Klíčová slova: gleosporiová hniloba; jablka; produkce etylenu; headspace gas analýza; odrůdy

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