

Ethylene Production, Tissue Senescence and Local Virus Infections

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Ethylene promotes the expression of one of the most common symptoms of ageing: the suppression of protein and nucleic acid synthesis. Artificial inhibition or promotion of protein synthesis results in an increased or decreased evolution of ethylene, respectively. In hosts with viral local lesions caused by TMV and TNV, a certain degree of enhanced ethylene production was demonstrated before the appearance of local lesions. However, the mass of the evolution of ethylene occurred at the very beginning of tissue necrosis. In systemic infections the rate of ethylene production remained unchanged. In another local virus infection (bean yellow mosaic virus on *Tetragonia expansa*), causing chlorotic spots instead of necroses, the evolution of ethylene also increased. It is concluded that the increased production of ethylene is connected to local tissue senescence caused by local virus infections but not necessarily to local necroses.

Our previous experiments (BALÁZS, GÁBORJÁNYI, TÓTH and KIRÁLY, 1969) have shown that the production of ethylene — a hormone of plant senescence — markedly increases as a result of virus infection in a local lesion host. This finding has been supported by similar results of NAKAGAKI, HIRAI and STAHMANN (1970). The increased production of ethylene is associated with tissue necrosis (local lesion production) induced by virus infections. This is the conclusion of our experiments mentioned above in which we infected a single cultivar of tobacco (namely Xanthi-nc) with two viruses, one of which induces local lesions in infected leaves (tobacco mosaic virus) and another which spreads systemically in the tissues without tissue necrosis (cucumber mosaic virus). Ethylene increases *only* if the host is infected with tobacco mosaic virus (TMV), however, ethylene production does not change in the *same cultivar* if it serves as a systemic host for cucumber mosaic virus (CMV). This finding proved to be valid also for another local virus infection, associated with tissue necrosis: in recent experiments we provided evidence for increased ethylene production in Pinto bean leaves infected with tobacco necrosis virus (TNV).

Role of Ethylene in Plant Senescence

As regards local lesions (tissue necroses) induced by viruses, our previous experience has shown that local lesion development is promoted if tissue senescence is enhanced in the host by different means (KIRÁLY, EL HAMMADY and

POZSÁR, 1968). On the other hand, local lesions are suppressed if juvenility is promoted in the host by applying cytokinin-type hormones or by removing the terminal bud. Additional support for this idea came from the experiments of OPEL (1965) and recently by NAKAGAKI and MATSUI (1971). All these findings show that the development of local lesions (necroses) is somehow connected to the ageing of host tissues.

Table 1

The effect of ethylene on the incorporations into the TCA insoluble fraction of Pinto bean leaves (cpm/g fr. wt.)

	Control	Ethylene 10 ³ ppm
Methionine-C ¹⁴	3505	465
Orotic acid-C ¹⁴	445	180

Table 2

The effect of treatments promoting or suppressing protein synthesis on the ethylene production in Pinto bean plants

Treatment	Ethylene (μ l/g) day	Per cent
Control	0.78	100
Chloramphenicol (200 ppm)	1.13	145.0
Fluorophenylalanine (100 ppm)	1.58	202.5
Puromycin (50 ppm)	1.11	142.0
Cycloheximide (10 ppm)	0.72	92.0
Heat treatment (50°C, 20 sec.)	1.15	275.5
Benzyladenine (30 ppm)	0.42	58.6
Decapitation	0.49	63.1

The phytoogerontological effect of ethylene was proposed by HALLAWAY and OSBORNE (1969) and, recently, undoubtedly demonstrated by ABELES, CRAKER and LEATHER (1971). Accordingly, these workers believe that ethylene is required for the process of ageing. Similarly, we were able to show that ethylene markedly inhibits protein and nucleic acid synthesis in leaves. This result is summarized in Table 1, showing an inhibition of incorporation of precursors of protein and RNA into the TCA-insoluble fraction of Pinto bean leaves. Thus, ethylene seems to promote the expression of one of the most common symptoms of ageing: the suppression of protein and nucleic acid synthesis.

It is of interest that artificial inhibition of protein synthesis results in the yellowing of leaf tissues. This is a typical senescence effect, which is in connection with increased ethylene production (Table 2). On the other hand, both ethylene

and ageing are suppressed by treatments causing juvenility in plants (decapitation or treatment with the cytokinin benzyladenine). This is the conclusion of experiments summarized in Table 2. Primary leaves of Pinto bean plants were brushed daily for 5 days with antibiotics inhibiting protein synthesis or with benzyladenine which stimulates the syntheses in plants causing, thereby, a juvenility effect. Decapitation was made at the appearance of the first trifoliate leaf. Heat treatment was performed by immersing primary leaves of Pinto bean in hot water (50°C) for 20 min. Ethylene production was measured gas-chromatographically as described earlier (BALÁZS, GÁBORJÁNYI, TÓTH and KIRÁLY, 1969). The results indicate that protein inhibitors promote the production of ethylene as well as the process of ageing. One apparent exception is cycloheximide. This antibiotic although inhibiting protein synthesis does not induce ethylene production. Senescence (yellowing) of leaves does not occur in this case, which may be a reasonable consequence of the unchanged level of ethylene. As is seen, the promotion of juvenility suppress ethylene as well as ageing in the leaves. Consequently, the data presented here suggest that ethylene is connected to plant senescence. As regards the origin of ethylene, this is still a disputed question.

Very recently, DEMOREST and STAHMANN (1971) proposed a model for the production of ethylene in plants from peptides and protein containing methionine. According to their suggestion proteolysis in plants may increase ethylene production by producing peptides with a C-terminal methionine residue. Peptides of this type produce ethylene in a peroxidase system at significant rates. It has been reported that oxidases are activated in virus-infected plants (FARKAS, KIRÁLY and SOLYMOSSY, 1960, VAN LOON, 1971, see the first paper in this volume, pp. 9—20). However, an increase in proteolytic activity during local virus infections has not been observed as yet, although one can suppose that local yellowing or local senescence is connected with a certain degree of proteolysis. This remains to be demonstrated in further experiments.

Role of Ethylene in Local Lesion Production

We supposed that local necrotic spots induced by TMV could be the consequence of an enhanced local senescence in host leaf tissues caused or followed by ethylene production. Supporting the first possibility it would be the demonstration of an enhanced production of ethylene *before* the appearance of local lesions. Accordingly, we measured ethylene production in Xanthi tobacco leaves infected with TMV in 3 hr intervals. We infected a series of uniform plants in the greenhouse and samples have been taken at 21, 24, 27, 30 and 33 hr after infection for determining the evolution of ethylene. Experimental methods of measuring ethylene production were described earlier (BALÁZS, GÁBORJÁNYI, TÓTH, and KIRÁLY, 1969). As is seen in Fig. 1 ethylene production increased at the very beginning of tissue necrosis in the case of local lesion infection (TMV). A certain degree of enhanced

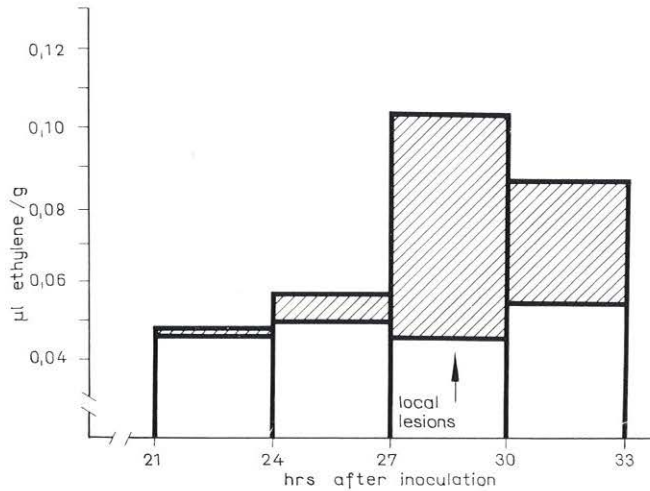


Fig. 1. Ethylene production in Xanthi tobacco leaves infected with TMV. Open bars: uninfected, solid bars: infected

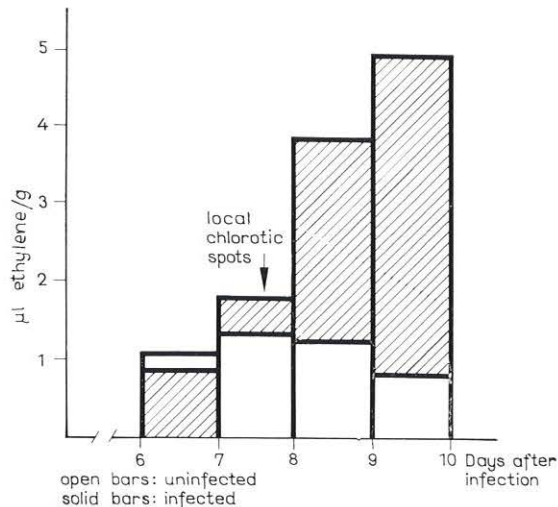


Fig. 2. Evolution of ethylene in *Tetragonia expansa* infected with bean yellow mosaic virus. Open bars: uninfected, solid bars: infected

ethylene production was demonstrated before the appearance of local lesions. However, this enhancement is not enough convincing. An impressive increase in the evolution of ethylene occurs somewhat later: at the beginning of lesion development. Thus, our endeavour to demonstrate a cause-and-effect relationship of ethylene with local senescence or local necrosis resulted in uncertain conclusion.

It still remained the possibility that the increased ethylene production perhaps was the consequence and not the cause of tissue decomposition (necrosis).

To gain a deeper insight into this mechanism we investigated the ethylene production in the case of another local virus infection causing chlorotic spots instead of necrosis. In the host-parasite combination of *Tetragonia expansa* and bean yellow mosaic virus the chlorotic spots of infected leaves represent an advanced degree of tissue senescence, and still, without tissue necrosis (without local lesions). As is seen in Fig. 2 ethylene production was increased also in this host-parasite combination at the time of the appearance of chlorotic local spots. On the basis of this result one can conclude that the increased ethylene production of the local lesion hosts is not the consequence of the development of tissue necrosis.

In summary, on the basis of our experimental data it is not possible to postulate unequivocally that viral lesions are the results of an increased ethylene production. On the other hand, it is also true that viral lesions are not causing an increase in the production of ethylene. We believe that the experiments described here present evidence for the association of local virus infections with local senescence and with an increased evolution of ethylene.

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