Ozone and ethylene stress

SIR—In a recent News and Views article[1] "Adding ethylene to injury", M. Unsworth discussed the results of Mehlhorn and Wellburn[2], who reported that both endogenous ethylene (a stress-hormone) and exogenous ozone are co-operating prerequisites for injury to pea plants. This important experimental result is indeed a milestone in plant toxicology. But Unsworth's statement that a co-operation of ozone and ethylene in producing plant damage... "does not seem to have been suggested previously..." needs some comments.

In 1985, we reported[3] changes in anti-oxidative activities in damaged spruce needles correlated with the ethylene precursor ACC or malonyl-ACC as a stress marker. We concluded that reaction products of the interaction between ozone and ethylene, namely peroxides and reactive aldehydes, might be the damaging species. We summarized these conclusions in the simplified equation:

$$O_3 + C_2H_4 + H_2O \rightarrow 2HCHO + H_2O_2$$

The basis of this theory was published in 1984 in German[4]. In later publications[5,6] we state that "the primary damaging reactions in spruce needles may operate as follows: (1) Trees under 'stress' produce the plant hormone ethylene; (2) ethylene and ozone react extremely fast, forming hydrogen peroxide and formaldehyde, compounds which may damage the wax layer; (3) ozone as a very aggressive..." (from ref.6). The findings of Mehlhorn and Wellburn with peas justify our earlier assumptions on forest decline and are therefore of utmost importance.

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1. Unsworth, M. *Nature* **327**, 364–365 (1987).
2. Mehlhorn, H. & Wellburn, A.R. *Nature* **327**, 417–418 (1987).
3. Elstner, E.F., Osswald, W. & Youngman, R.J. *Experientia* **41**, 591–597 (1985).
4. Elstner, E.F. & Oswald, W. *Naturw. Rundschau* **37**(2), 52–61 (1984).
5. Osswald, W. & Elstner, E.F. *Ber. Deutsch. Bot. Ges.* **99**, 313–339 (1986).
6. Osswald, W. & Elstner, E.F. *Free Radical Res. Commun.* **3**, 185–192 (1987).

Sequence specificity of retroviral proteases

SIR—The protease encoded by retroviruses cleaves the *gag*-polyprotein to produce the amino terminus of the major core protein (p24, p27 or p30, depending on the virus)[1-3]. On examining the amino-acid sequence that spans this cleavage site in available retroviral sequences we have found the strongly conserved pattern X-Y-Pro-Z, where X is generally small with some hydrophobic preference, Y is aromatic or large and hydrophobic, and Z is small and hydrophobic (Fig. 1). The totally conserved proline forms the amino terminus of the major core protein. Some weaker clusters of conservation are also seen in the flanking residues.

Fig.1 Alignment of sequences in retroviral *gag*-polyproteins, which are cleaved to yield the N-terminal proline of the major core protein. Sequences are from mouse mammary tumour virus (MMTV), hamster intracisternal A-particle (HIAP-18), simian AIDS retrovirus (SRV-1), human T-cell leukaemia virus type II (HTLV-II), AKV murine leukaemia virus (AKV-MuLV), Moloney murine leukaemia virus (Mo-MuLV), feline leukaemia virus (FeLV), H7-baboon endogenous virus (H7BEV), human immunosupressive virus type 1 (HIV-1), human immunosupressive virus type 2 (HIV-2), visna lentivirus (VLV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FSV), human T-cell leukaemia virus type I (HTLV-I), feline sarcoma virus (FeSV), simian sarcoma virus (SSV) and bovine leukaemia virus (BLV).

MMTV	Thr Phe Thr Phe **Pro** Val Va	
HIAP-18	Gln Met Ala Phe **Pro** Val Ph	
SRV-1	Lys Asp Ile Phe **Pro** Val Th	
HTLV-II	Thr Gln Cys Phe **Pro** Ile Le	
AKV-MuLV	Ser Arg Ala Phe **Pro** Leu Ar	
Mo-MuLV	Ser Gln Ala Phe **Pro** Leu Ar	
FeLV	Ser Gln Ala Phe **Pro** Leu Ar	
H7BEV	Ser Ser Leu Phe **Pro** Leu Ar	
HIV-1	Ser Gln Asn Tyr **Pro** Ile Va	
HIV-2	Gly Gly Asn Tyr **Pro** Val Gl	
VLV	Arg Glu Val Tyr **Pro** Ile Va	
RSV	Val Val Ala Met **Pro** Val Va	
FSV	Met Val Ala Met **Pro** Val Va	
HTLV-I	Pro Ala Ile Leu **Pro** Ile Il	
FeSV	Ser Gln Ala Leu **Pro** Leu Ar	
SSV	Thr Val Ile Leu **Pro** Leu Ar	
BLV	Pro Ala Ile Leu **Pro** Ile Il	

AKV-MuLV	Ser Ala Leu Tyr **Pro** Ala Leu	p15/p12	*gag*
Fr-SFFV	Ser Ala Leu Tyr **Pro** Ala Leu		
FeLV	Ser Ser Leu Tyr **Pro** Ala Leu		
Mo-MuLV	Ser Ser Leu Tyr **Pro** Ala Leu		
FeSV	Ser Ser Leu Tyr **Pro** Val Leu		
EqIAV	Ser Glu Glu Tyr **Pro** Ile Met		
SSV	Pro Pro Ile Tyr **Pro** Ala Thr		
RSV	Phe Gln Ala Tyr **Pro** Leu Arg	RT/enuc	*pol*
HIV-1	Thr Leu Asn Phe **Pro** Ile Ser	Prt/RT	*pol*
HIV-2	Ser Leu Asn Leu **Pro** Val Ala		

Fig. 2 Alignment of othe sequences in viral polyprotein which are cleaved to yield N termini and which may be sub strates for the virally encode proteases. Sequences are fro AKV-MuLV, Friend splee focus-forming virus (Fr-SFFV FeLV, Mo-MuLV, FeSV equine infectious aneamia viru (EqIAV), SSV, RSV, HIV- and HIV-2.

An additional closely related pattern of sequences is found in some *gag*-polyprotein sequences (Fig. 2), corresponding to the junctions of the p15 and p12 proteins, which may also be a site for cleavage by the viral protease. In the *pol*-polyprotein sequence of Rous sarcoma virus, a sequence matching this pattern occurs at the junction of the reverse transcriptase and the endonuclease, while in the AIDS viruses HIV-1 and HIV-2, a matching sequence occurs at the junction of the (presumed) carboxy terminus of the protease sequence itself with the amino-terminus of the reverse transcriptase, implying that the production of active reverse transcriptase in these viruses may well be dependant upon the action of the virally encoded protease. No matches with this sequence pattern are found at known cleavage sites in the various *env*-polyproteins. It is likely that this overall pattern represents the preferred amino-acid sequence for cleavage by retroviral proteases and may be of use in the design of specific inhibitors of retroviral protease activity for the chemotherapy of AIDS.

Observations that retroviral proteases may be inhibited by thiol-specific reagents[4,5] has led to suggestions that these enzymes might be thiol-proteases[6]. A more related sequences have becom available this hypothesis has appeare more unlikely since the position an number of cysteine residues are found t be poorly conserved. In the recently pub lished[7] sequence of HIV-2, we have ident fied the likely position of the amino te minus of the protease as close to residu 85, with the carboxy terminus immediate ly preceding the probable amino terminu of the reverse transcriptase close to res due 185 (residue numbers are relative t the start of the *pol* open reading frame) No cysteine residues occur within thi sequence.

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1. Yoshinaka, Y., Shames, R.B., Luftig, R.B., Smythe G.W. & Oroszlan, S. *J. gen. Virol.* **66**, 379-383 (1985).
2. Yoshinaka, Y., Katoh, I., Copeland, T.D., Smythers, G. & Oroszlan, S. *J. Virol.* **57**, 826-832 (1986).
3. Kramer, R.A. *et al. Science* **231**, 1580-1584 (1986).
4. Yoshinaka, Y. & Luftig, R.B. *Cell* **12**, 709-719 (1977).
5. Dittmar, K. & Moelling, K. *J. Virol.* **28**, 106-118 (1987).
6. Yoshinaka, Y., Katoh, I., Copeland, T.D. & Oroszlan. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1618-1622 (1985).
7. Guyader, M. *et al. Nature* **326**, 662-669 (1987).