condition which may reduce the sexual receptivity of females is to subject them to continuous light[6]. Such females showed a slight discrimination against the spotless males in the no-choice experiment ($p < 0.05$), though there were no significant differences in the average time of copulation. More significant reduction in the mating success of the amputated males as compared with the intact males was found with the female choice experiment ($p < 0.01$). Again, the spotless males mated as quickly as normal males did. The time course of the female choice experiment is graphically presented in the figure.

These results indicate that the visual stimulus produced by the male wing spots of *D. suzukii* interact with many other stimuli to enhance the sexual receptivity of females, which was generally shown in the courtship stimuli of *Drosophila*[7]. The effect of deprivation of this particular stimulus could be detected only when the female's threshold of receptivity was high. It may also be suggested that the male's specific character of the black spot has been evolved and maintained for their sexual selection.

1 H.T. Spieth, A. Rev. Entomol. *19*, 385 (1974).
2 A.W. Ewing, Physiol. Entomol. *3*, 33 (1978).
3 J. Grossfield, Behav. Genet. *2*, 311 (1972).
4 A. Manning, Viewpoints Biol. *4*, 125 (1965).
5 A.W. Ewing, Anim. Behav. *12*, 316 (1964).
6 R. Hardeland, Anim. Behav. *20*, 170 (1972).
7 H.C. Bennet-Clark, M. Dow, A.W. Ewing, A. Manning and F. von Schilcher, Behav. Genet. *6*, 93 (1976).

Gibberellins and the break of bud dormancy in virus-infected stem cuttings of *Euphorbia pulcherrima*

S. Nath, C.L. Mandahar and A. Gulati

Department of Botany, Panjab University, Chandigarh 160014 (India), 19 December 1978

Summary. Break in bud dormancy in virus-infected stem cuttings of *Euphorbia pulcherrima* occurs because of the higher quantity of gibberellins present in them than in healthy cuttings in the dormant period of the plant.

During our studies of rooting stem cuttings of virus-infected *E. pulcherrima*, we found that the buds on these cuttings (when planted in sand in earthen pots and periodically irrigated with water) grew into leafy shoots during winter while the buds on identically treated healthy cuttings either remained dormant or grew into cyathia[1]. The virus thus breaks the dormancy of buds in infected stem cuttings. Induction and break in bud dormancy in plants is controlled by an interaction of growth promotors, particularly gibberellins, and the growth inhibitors abscisic acid and phenols[2-5]. The role of growth inhibitors in breaking bud dormancy in virus-infected *E. pulcherrima* has already been reported[5], while the role of gibberellins is reported here.

The conclusion that gibberellins break the dormancy of many woody plants is based on the estimation of endogenous gibberellins at different periods of the year[2,6-8]. Such estimations show that the quantity of gibberellins in plants decreases during their dormant period[2,4,7]. Estimation of endogenous gibberellins was done twice a year to see if gibberellins play some part in breaking bud dormany in *E. pulcherrima*.

Material and method. Gibberellins were extracted from the 150 g of bark of stem cuttings by the method of West and Phinney[9] and MacMillan et al.[10]. The residue so obtained was dissolved in 10 ml distilled water which was then employed for the dwarf pea seedling bioassay according to the method of Radley[11] and Radley and Dear[12]. The increase in shoot length of 20 pea seedlings was measured from the cotyledonary node to the uppermost node after seven days of treatment. The gibberellin activity is expressed in percentage increase in shoot length of pea seedlings over the control (untreated pea seedlings) and is given in the table.

Results and discussion. All the water-treated diseased cuttings showed bud sprouting while buds on water-treated healthy cuttings either remained dormant or rarely grew into cyathia.

Dwarf pea seedling bioassay for the estimation of gibberellin-like activity in the purified extracts of healthy and diseased stem tissues of *E. pulcherrima*

Month	Treatment	Percentage average shoot length (in cm) of the pea seedlings after 7 days	Percentage increase in shoot length as compared to control (water)	Percentage increase (+) or decrease (−) in shoot length of pea seedlings on application of diseased extract with respect to healthy extract
	Control (H_2O)	5.42±0.1559*	-	-
May (growing period)	H-gibberellin extract	11.36±0.3000*	109.59	-
	D-gibberellin extract	8.84±0.1163*	63.09	22.18 (−)
December (dormant period)	H-gibberellin extract	5.72±0.2234*	5.53	-
	D-gibberellin extract	6.50±0.1225*	19.92	13.63 (+)
	Standards			
	0.01 ppm GA_3	5.50±0.177*	1.47	-
	0.1 ppm GA_3	6.72±0.214*	23.98	-
	1 ppm GA_3	7.50±0.066*	38.37	-
	10 ppm GA_3	11.50±0.177*	112.17	-

H = The gibberellin extract of healthy stem tissue. D = The gibberellin extract of diseased stem tissue. * = SE.

Gibberellin activity was noted twice a year, once in the growing season (May) and once in the dormant period (December) of the plant (table). The gibberellin activity in both healthy and diseased cuttings was high in May but decresaed greatly in December (to 5.53% in healthy and to 19.92% in diseased cuttings in terms of percentage average shoot length in cm). Thus while gibberellin activity in May was 22.18% less in diseased cuttings than in the healthy ones but in December the gibberellin activity was 13.63% higher in diseased than in healthy cuttings.

It appears from above that the higher quantity of gibberellins in diseased cuttings in December could be responsible for the break in bud dormancy of virus-infected stem cuttings of *E. pulcherrima* during the dormant season of the plant. This becomes all the more significant when these results are viewed in the context that, as already reported[5], the amounts of growth inhibitors (abscisic acid and phenols) present in diseased stem cuttings during the dormant period of the plant was less than in the healthy cuttings. It may also be mentioned that the pattern of changes of gibberellins in *E. pulcherrima* during the growing and dormant seasons was found to be the same as has already been reported widely[2,6,7]; that is, the amount of gibberellin decreases in plants with the onset of the dormant season. The degree of decrease in diseased plants (as shown above), however, is less so that the amount of gibberellins left behind in diseased stem cuttings are enough for the vegetative growth of the buds on them. The smaller amount of inhibitors in diseased cuttings also appears to be helpful in this connection.

Virus-infected *E. pulcherrima* seems to be the first virus-host combination in which studies of the break in bud dormancy, and estimations of endogenous growth regulators and growth inhibitors has been carried out. However, the role of gibberellins in the stunting of plants infected by mycoplasma-like organisms has been reported in only one study[13] while the physiological basis for this has been theoretically discussed[14]. The virus-host combination investigated by us may prove an ideal system in this connection.

1 A. Gulati, M.Sc. Thesis, Panjab University, Chandigarh, India (1976).
2 P.F. Wareing and P.F. Saunders, A. Rev. Pl. Physiol. *22*, 261 (1971).
3 V.I. Kefli and C.Sh. Kadyrov, A. Rev. Pl. Physiol. *22*, 185 (1971).
4 Y. Tsukamoto and H. Konoshima, Physiol. Pl. *26*, 244 (1972).
5 C.L. Mandahar, S. Nath and A. Gulati, Experientia *35*, 315 (1979).
6 P.F. Wareing, Soc. exp. Biol. Symp. *23*, 241 (1969).
7 A. Alpi, N. Ceccarelli, F. Tognoni and G. Gregorini, Physiol. Pl. *36*, 362 (1976).
8 I.D. Railton and P.F. Wareing, Physiol. Pl. *28*, 88 (1973).
9 C.A. West and B.O. Phinney, J. Am. chem. Soc. *81*, 2424 (1959).
10 J. MacMillan, J.C. Seaton and P.J. Suter, Adv. Chem. *28*, 18 (1961).
11 M. Radley, Ann. Bot. *22*, 297 (1958).
12 M. Radley and E. Dear, Nature *182*, 1098 (1958).
13 K. Maramrosch, Science *126*, 651 (1957).
14 C.L. Mandahar, Introduction to Plant viruses, p.333. S. Chand & Co., New Delhi 1978.

Changes of X-prolyl dipeptidyl-aminopeptidase activity in developing rat brain

T. Kato, T. Hama, T. Nagatsu, H. Kuzuya and S. Sakakibara

Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuta, Tokyo Institute of Technology, Yokohama 227 (Japan); Department of Biochemistry, Tohoku Dental University, Koriyama, Fukushima 963 (Japan); and Peptide Institute, Protein Research Foundation, Minoh, Osaka 562 (Japan), 22 January 1979

Summary. We found X-prolyl dipeptidyl-aminopeptidase activity in rat brain and examined the developmental changes at various ages. The total enzyme activity per brain increased until 4 weeks of age, and then decreased during maturation. Specific activity in young rat brain was higher than that in adult rat brain. The properties of the brain enzyme were different from those of pituitary and other tissues.

Protein turnover is highly active in the brain, and most proteins are in a dynamic state. Although changes of proteolytic enzymes in developing rat brain were reported by a few groups[1-3], the physiological role of the peptidases is not known. In our laboratory the physiological roles of X-prolyl dipeptidyl-aminopeptidase, which was discovered in rat liver and kidney by Hopsu-Havu and Glenner[4], have been studied in various tissues for the last several years[5-8]. Recently we found that X-prolyl dipeptidyl-aminopeptidase purified from human submaxillary gland[9] hydrolyzed N-terminal dipeptide Arg-Pro and subsequent dipeptide Lys-Pro from substance P[10]. Interestingly, the N-terminal tetrapeptide Arg-Pro-Lys-Pro-OH of substance P was shown to have almost the same effect as substance P on the neurite extension of neuroblastoma N-18 cells[11], and the N-terminal dipeptide Arg-Pro of substance P, which are cleaved by the enzyme, also had the same effect but neither arginine nor proline alone had the effect (Narumi and Maki, personal communication). We have also found X-prolyl dipeptidyl-aminopeptidase activity in human cerebrospinal fluid[8], suggesting the presence of the enzyme in the brain. The enzyme in the brain may release N-terminal dipeptide Arg-Pro from some brain peptides such as substance P and bradykinin. In this paper we report the presence, some properties and the changes during development of rat brain, of X-prolyl dipeptidyl-aminopeptidase activity.

Materials and methods. p-Nitroanilides (pNA) of Arg-Pro, Lys-Pro, Gly-Pro, Ala-Ala, Gly-Ala, Ala-Gly and Gly-Leu, and 7-(Gly-Pro)-4-methylcoumarinamide (Gly-Pro-MCA) were synthesized at Protein Research Foundation (Minoh, Osaka, Japan) as reported previously[7,12,13]. Sprague-Dawley rats were raised in our laboratory. At each age, the rat was killed by decapitation, and the brain was quickly removed, frozen and stored at −80 °C. The brain was homogenized in 3 vol. of 0.25 M sucrose. Enzyme activity of the brain homogenate at various ages was assayed with Gly-Pro-MCA as reported previously[13], except using Tris-maleate buffer, pH 7.0. The incubation mixture (total vol. 100 µl) contained 20 mM Tris-maleate buffer, pH 7.0, 0.5 mM Gly-Pro-MCA tosylate and enzyme. The incubation was done at 37 °C for 30 min and the reaction was stopped by adding 1.0 ml of 1 M sodium acetate buffer (pH 4.2). After centrifugation, the fluorescence intensity of 7-amino-4-methylcoumarin liberated by the enzyme reaction was measured at 460 nm with excitation at 380 nm. To