Scientia Horticulturae, 21 (1983) 291—297
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

IDENTIFICATION OF THE ETHYLENE PRECURSOR, 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC), IN POLLEN

CHARLES S. WHITEHEAD[1], DAVID W. FUJINO and MICHAEL S. REID

Department of Environmental Horticulture, University of California, Davis, CA 95616 (U.S.A.)

[1] On leave from: Department of Botany, Potchefstroom University for CHE, Potchefstroom, South Africa 2520

(Accepted for publication 15 June 1983)

ABSTRACT

Whitehead, C.S., Fujino, D.W. and Reid, M.S., 1983. Identification of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), in pollen. *Scientia Hortic.*, 21: 291—297.

Within 15 min after application of pollen to the stigma of carnation flowers (*Dianthus caryophyllus* L. cultivar 'White Sim'), ethylene (C_2H_4) production by the gynoecium had increased substantially. Pollen germination did not start until 1 h after pollination. Analysis of materials removed from pollen by a brief rinse with an aqueous solvent showed the presence of high concentrations of the C_2H_4 precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). This compound, which was found to be present in pollen from a number of flower species and which increased in concentration as the anthers developed, may be an important mediator of the early response of flowers to pollination.

Keywords: 1-aminocyclopropane-1-carboxylic acid; carnation; ethylene; gynoecium; pollen.

INTRODUCTION

The common observation that pollination of flowers results in early wilting of the petals has been suggested to be a response to auxin liberated during the post-pollination period (Burg and Dijkman, 1967) or to mechanical injury as a result of penetration of the stylar tissues by the growing pollen tube (Gilissen, 1977). The wilting of carnation flowers following pollination with pollen from "miniature" carnations is accompanied by a surge of C_2H_4 produced from the gynoecium and the petals (Nichols, 1977). Since ACC is the immediate precursor to C_2H_4 in higher plants (Adams and Yang, 1979), we examined the ACC content in various plant organs of pollinated carnation flowers. We found a sequence of changes which suggested that ACC might be involved in coordinating the events following pollination (Nichols et al., 1983). In further investigating this hypothesis, we carried out experiments to examine early events in the

0304-4238/83/$03.00

post-pollination period. We here report evidence that the pollen carries to the flower sufficient ACC to explain the early stimulation of C_2H_4 production by the gynoecium.

MATERIALS AND METHODS

Plant materials. — Flowers were grown in the greenhouse at Davis using normal commercial practices. Pollen was obtained from freshly dehisced anthers as previously described (Nichols et al., 1983).

C_2H_4 production by pollinated stigmas. — Pollen of the 'Exquisite' miniature carnation cultivar was applied to the stigmas of 'White Sim' standard carnations as described by Nichols et al. (1983). The production of C_2H_4 by the gynoecia of the intact flowers was measured by aspirating C_2H_4-free air at 1 l h^{-1} through a glass tube placed over the entire gynoecium (ovary, styles and stigmas) and measuring the C_2H_4 content in samples of the air stream using gas chromatography (Bufler et al., 1980).

Pollen tube growth. — The relationship between increased C_2H_4 production by gynoecia of pollinated flowers and pollen germination was examined by removing the stigmas from pollinated flowers at intervals for 6 h after pollination. The styles were autoclaved (200 kPa, 120°C, 20 min) in 5% Na_2SO_3, then squashed in 0.005% decolourized aniline blue and examined by epifluorescence microscopy (Carl Zeiss, Jena). Germinated pollen grains were identified by the blue fluorescence of the callose around the pollen tube (Shivanna et al., 1978).

Extraction, measurement and identification of ACC. — ACC was extracted from pollen by briefly rinsing (1 min) approximately 10 mg of pollen obtained from freshly dehisced anthers with a standard pollen germination medium containing 10% sucrose and 0.01% H_3BO_4. The suspensions were Millipore filtered (pore size 0.45 μm) and assayed for ACC by oxidation with alkaline NaOCl and gas chromatographic determination of the liberated C_2H_4 (Bufler et al., 1980).

To verify the nature of the C_2H_4-liberating material on the pollen, a sample of carnation pollen was extracted, filtered, then purified by eluting from a Dowex 50 ion exchange column (H^+ form). The eluate was analyzed by paper chromatography (descending, butanol:acetic acid: water, 4:1:5, upper phase) and the material co-chromatographing with authentic ACC was then further analyzed by paper electrophoresis (pH 2, acetic acid, 2000 V, 1 h).

Changes in ACC content of developing anthers. — Anthers were dissected from buds at different stages of development (Fig. 3). and their ACC content was measured as previously described (Bufler et al., 1980).

RESULTS

Effect of pollination on C_2H_4 production. — The C_2H_4 production of gynoecia from pollinated flowers was found to be significantly higher than that of control gynoecia at the earliest time measured (15 min after pollination) (Fig. 1). Thereafter, C_2H_4 production of pollinated gynoecia increased exponentially while that of control gynoecia fell below detectable levels.

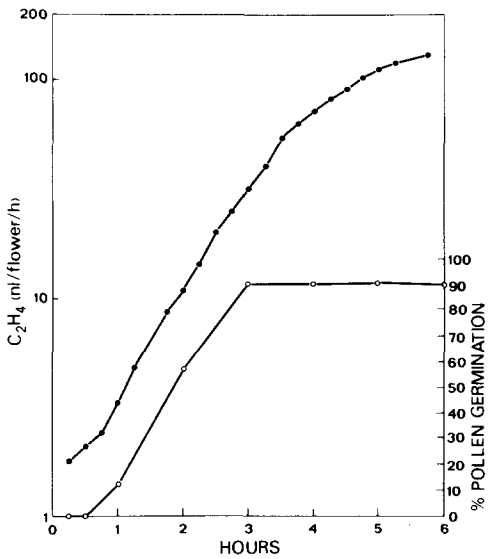

Fig. 1. Post-pollination changes in carnation gynoecia. Standard carnation flowers ('White Sim') were pollinated with pollen from a miniature carnation and their production of C_2H_4 was measured while they were still attached to the flower. At intervals, stigmas were removed from replicate flowers and the percentage of pollen grains which had germinated was determined. •—•, C_2H_4 production by the gynoecium; ○—○, percent germinated pollen grains.

Germination of pollen on carnation stigmas. — Examination of stylar squashes from pollinated flowers showed that the pollen did not germinate until 1 h after pollination (Fig. 1). Ninety percent of the pollen had germinated after 3 h.

Identification of ACC in pollen. — The ACC content of pollen from the different flower species ranged from 0.6 nmol g^{-1} for cornflower to 296 nmol g^{-1} for petunia (Table I). Compounds in the filtrate from a suspension of carnation pollen in 10% sucrose and 0.01% H_3BO_4 were separated by paper chromatography. Elution of 2.0-cm zones from the chromatograms with 50% aqueous methanol revealed the presence of material which could be oxidized to C_2H_4 at the R_f (0.39) of authentic ACC

TABLE I

ACC content of pollens from different flowers. Pollen was removed from freshly dehisced anthers of flowers, extracted, filtered and assayed for ACC as described in the text

Pollen source	ACC content (nmol g^{-1})
Petunia hybrida (petunia)	296.0
Lathyrus odoratus (sweet pea)	50.0
Dianthus caryophyllus (carnation)	22.8
Gerbera jamesonii (Transvaal daisy)	11.4
Cucurbita maxima (zucchini squash)	4.6
Papaver nudicaule (Iceland poppy)	1.8
Anthirrhinum majus (snapdragon)	1.7
Centaurea cyanus (cornflower)	0.6

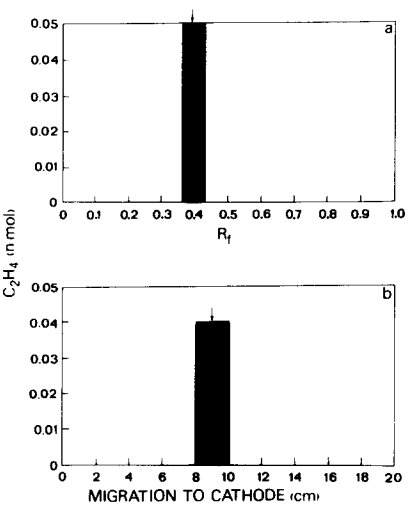

Fig. 2. Histograms of C_2H_4 produced by oxidation of eluates obtained from excised zones after (a) paper chromatography and (b) paper electrophoresis of carnation pollen extracts. The arrows indicate the location of authentic ACC.

(Fig. 2a). When the eluted material was further analyzed by paper electrophoresis, the C_2H_4-generating compound was again found in the same region as authentic ACC (Fig. 2b). The eluate of this region of the electrophoretogram contained 80% of the activity in the original extract.

Changes in ACC content of developing anthers. — ACC was present in anthers of 'Exquisite' miniature carnations even in very young buds (Fig. 3). During bud development, the concentration of ACC rose 5-fold, reaching a plateau as the flower started to open.

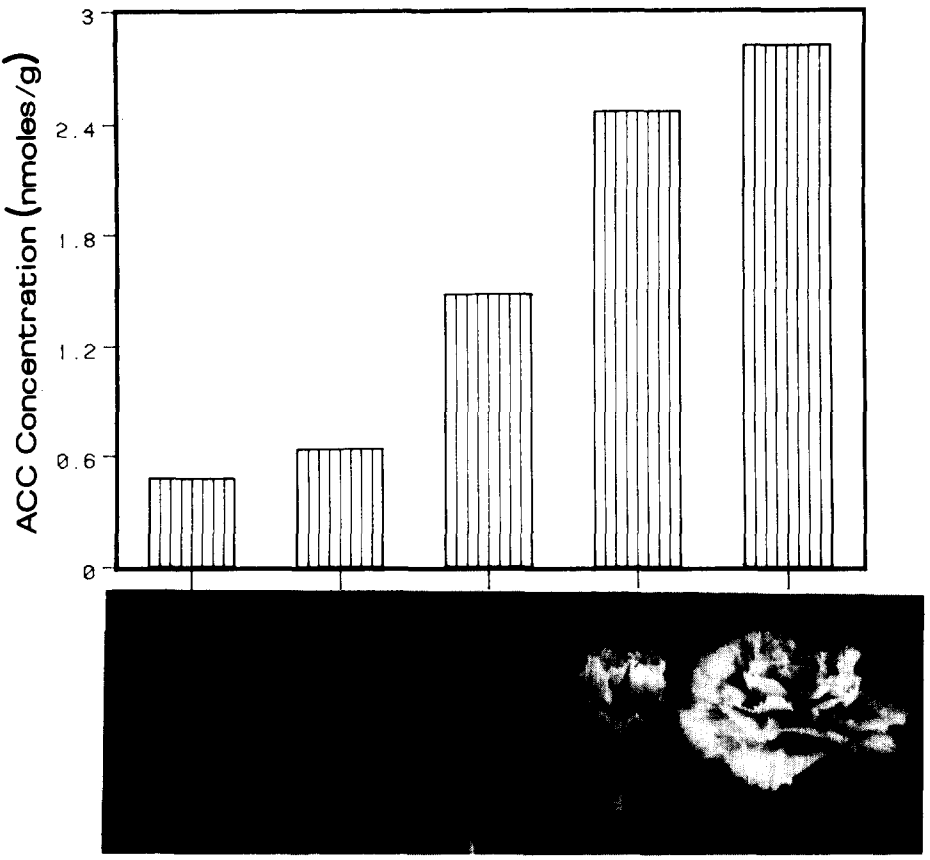

Fig. 3. Changes in ACC content of developing anthers. The ACC concentration in anthers dissected from buds of the sizes shown was measured as described in the text.

DISCUSSION

It is well known that pollination stimulates C_2H_4 production by certain flowers (Hall and Forsyth, 1967; Nichols, 1977) and that the post-pollination phenomena can be stimulated by C_2H_4 treatment of flowers (Nichols, 1971; Arditti et al., 1973). In the studies reported here, we showed that C_2H_4 production by the gynoecia increased markedly before any detectable pollen germination. This indicates that the initial response of the flower to pollination must either be to a chemical on the surface of the pollen, or to some "contact stimulus". Other workers have shown that the exine of pollen contains substantial concentrations of readily extractable amino acids (Linskens and Schrauwen, 1969), and it seemed possible that the amino acid precursor to C_2H_4, ACC, could also be present on the pollen. This would provide a good explanation for the early increase in C_2H_4 production by the gynoecia of pollinated flowers.

The extract obtained by briefly rinsing pollen with a pollen germination medium did liberate C_2H_4 in the standard oxidative assay for ACC. Recently, Savidge et al. (1983) have questioned the validity of this assay as an identification tool in studies of ACC. After chromatography and then electrophoresis of the pollen extract, the C_2H_4-liberating activity had the same R_f and mobility, respectively, as authentic ACC (Fig. 2a, b), which would appear to be adequate confirmation of the nature of the extracted material.

Because of the ease with which ACC can be removed from the pollen, it seems highly probable that it is located on the exine. The presence of ACC in the pollen of a number of plants (Table I) suggests that its presence in carnation pollen is not circumstantial, and that it may play an important role in the post-pollination phenomena. Moreover, the rapid rise in ACC content as the anthers matured (Fig. 3) seems to indicate that its synthesis is an integral part of pollen maturation.

Calculation of the expected contribution of pollen ACC to C_2H_4 production by pollinated carnations shows that there is enough ACC on the pollen to account for the C_2H_4 production measured during the first 30 min following pollination. We have already deduced the presence of high activities of the enzyme forming C_2H_4 from ACC in unpollinated carnation styles (Nichols et al., 1983). It would be interesting to see whether pollination of flowers with foreign pollen (for example petunia pollen on carnations) would result in an ephemeral production of C_2H_4 by the stigmas, and then whether this transient C_2H_4 production might have some effect on the stigma important to the growth of compatible pollen tubes. In pollination of watermelon (*Citrullus lanatus*), it has been demonstrated that major changes in ultrastructure of the stigmatic surface occur within 30 min of pollination (Sedgley, 1982). The liberation of C_2H_4 from ACC present in the pollen might be the signal which initiates these changes. The acceleration and continuation of C_2H_4 production by the gynoecia and petals thereafter are presumably a response to germination of the pollen tubes, and penetration and wounding of the stylar tissues, as suggested by Gilissen (1977).

ACKNOWLEDGEMENTS

We thank Dr. Neil Hoffman for assistance with the paper electrophoresis of ACC. C.S.W. was supported by a sabbatical leave grant from the C.S.I.R., South Africa.

REFERENCES

Adams, D.O. and Yang, S.F., 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci., 76: 170—174.

Arditti, J., Hogan, N.M. and Chadwick, A.V., 1973. Post-pollination phenomena in orchid flowers. IV. Effects of ethylene. Am. J. Bot., 60: 883—888.

Bufler, G., Mor, Y., Reid, M.S. and Yang, S.F., 1980. Changes in 1-aminocyclopropane-1-carboxylic acid content of cut carnation flowers in relation to their senescence. Planta, 150: 439—442.

Burg, S.P. and Dijkman, M.J., 1967. Ethylene and auxin participation in pollen-induced fading of Vanda orchid blossoms. Plant Physiol., 42: 1648—1650.

Gilissen, L.J.W., 1977. Style-controlled wilting of the flower. Planta, 133: 275—280.

Hall, I.V. and Forsyth, F.R., 1967. Production of ethylene by flowers following pollination and treatments with water and auxin. Can. J. Bot., 45: 1163—1166.

Linskens, H.F. and Schrauwen, J., 1969. The release of free amino acids from germinating pollen. Acta Bot. Neerl., 18: 605—614.

Nichols, R., 1971. Induction of flower senescence and gynoecium development in the carnation (*Dianthus caryophyllus*) by ethylene and 2-chloroethylphosphonic acid. J. Hortic. Sci., 46: 323—332.

Nichols, R., 1977. Sites of ethylene production in the pollinated and unpollinated senescing carnation (*Dianthus caryophyllus*) inflorescence. Planta, 135: 155—159.

Nichols, R., Bufler, G., Mor, Y., Fujino, D.W. and Reid, M.S., 1983. Changes in ethylene production and 1-aminocyclopropane-1-carboxylic acid content of pollinated carnation flowers. J. Plant Growth Regul., 2: in press.

Savidge, R.A., Mutumba, G.M.C., Heald, K.J. and Wareing, P.F., 1983. Gas chromatography—mass spectroscopy identification of 1-aminocyclopropane-1-carboxylic acid in compression wood vascular cambium of *Pinus contorta* Dougl. Plant Physiol., 71: 434—436.

Sedgley, M., 1982. Anatomy of the unpollinated and pollinated watermelon stigma. J. Cell Sci., 54: 341—355.

Shivanna, K.R., Heslop-Harrison, Y. and Heslop-Harrison, J., 1978. The pollen—stigma interaction: Bud pollination in the Cruciferae. Acta Bot. Neerl., 27: 107—119.