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DATE: March 4, 1985

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

ADMINISTRATIVE AND LEGAL COMMITTEE

Fifteenth Session
Geneva, March 27 and 28, 1985

PLANT VARIETY PROTECTION AND VIRUS DISEASES

Document prepared by the Office of the Union

The Office of the Union has received the letter given at the annex to this document. In agreement with the Chairman of the Committee, it is suggested that it be examined under item 9 ("Any other business") of the draft agenda.

[Annex follows]

Tel. (021) 26871

Department of Botany
University College
Cork

Professor:
A.C. Cassells, M.Sc., Ph.D.

Roinn na Luibheolaíochta
Coláiste na hOllscoile
Corcaigh

2 January 1985

Dr. Mast,
Chief Executive, UPOV,
Internationaler Verband zum Schutz von
Pflanzenzuechtungen,
34 Chemain des Colombettes,
1211 Geneva 20,
Switzerland.

Dear Dr. Mast,

Harlequin Pelargoniums

I am writing to you regarding the above after discussions with Frau I. Schumann of Pelargonien Fischer KG, Hillscheid-uber-Koblenz, West Germany.

I produced a wide range of picotee pelargoniums with my postgraduate student George Minas. These include all of those commercially released 'Harlequins' (see enclosed reprints). I understand from conversations at the Bundessortenamt in Hannover that difficulties have arisen with regard to the approval of these types for breeders' rights.

The Harlequins were produced by the exploitation of beneficial infectious agents and as such anticipate the use of genetically engineered virus gene-vectors. In this latter regard I am particularly anxious to press their case for plant breeders' rights in the understanding that UPOV may have to introduce new regulations to cover breeders' rights for genetically 'transformed' plants.

I believe it is important that breeders' rights be available to producers of 'transformed' plants to encourage the exploitation of biotechnology, while protecting growers and society in general from any possible risks. The main consideration here is that such agents are not spread naturally (i.e. in an uncontrolled fashion). Fortunately, the source of the picotee agent, 'Mexicana', has been grown extensively and widely for more than 10 years without, to my knowledge, any reports of uncontrolled spread. We have investigated this aspect extensively using various vectors etc.

A second feature of such agents is that they may resemble unstable genes, or some normal genes, in being affected predictably by environmental factors such as temperature. The picotee effect is influenced by temperature during early bud development.

I hope that you will respond positively to my suggestion that the Harlequins should be regarded as a test case for genetically transformed plants. I shall be pleased to clarify any matters you may raise and would welcome your advise as to how the case for breeders' rights might be progressed.

Happy New Year.

Yours sincerely,

A. C. Cassells

Enc.

[Reprints follow]

Scientia Horticulturae, 17 (1982) 89—96
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PELARGONIUM NET VEIN AGENT AND PELARGONIUM PETAL STREAK AS BENEFICIAL INFECTIONS OF COMMERCIAL PELARGONIUMS

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(Accepted for publication 3 August 1981)

ABSTRACT

Cassells, A.C., Minas, G. and Bailiss, K.W., 1982. Pelargonium net vein agent and pelargonium petal streak as beneficial infections of commercial pelargoniums. *Scientia Hortic.*, 17 (1982) 89—96.

Pelargonium Net Vein Agent (PNVA) and Pelargonium Petal Streak Agent (PPSA) have been shown to be graft-transmissable, singly and together, to both ivy-leaf and zonal pelargoniums. In zonal pelargoniums, PNVA symptoms are seasonal; PPSA results in petal curling in some cultivars and in most petal streaking is absent or only on the lower petal surface. PNVA symptoms were stable in the ivy-leaf cultivars studied, as were those of PPSA. There was no interaction between PPSA and PNVA, doubly-infected ivy-leaf cultivars showing both symptom types.

Symptoms induced by PNVA and PPSA were eliminated by meristem culture.

The commercial potential for the exploitation of PPSA and PNVA is discussed.

INTRODUCTION

Many commercially important pelargoniums ("geraniums") depend on abnormal pigment distribution, either in the leaves or flowers, for their commercial attractiveness (Clifford, 1970). The basis of these abnormalities is important for the micro-propagator, for beneficial infectious agents may be eliminated in meristem culture or chimeras broken down in callus culture (Cassells et al., 1980). Conversely, beneficially infected cultivars can be propagated via callus/explant culture and chimeras via meristem culture (Cassells et al., 1980).

The ivy-leaf pelargoniums ("geranium") cultivars 'Crocodile' (syn. 'Sussex Lace') (Fig. 1a) and 'Mexicana' (syn. 'Roulette') (Fig. 1b) are beneficially infected with graft-transmissible agents, pelargonium net vein agent (PNVA) and pelargonium petal streak agent (PPSA), respectively. A rhabdovirus has been detected in thin sections of ivy-leaf pelargoniums showing

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chlorotic vein-clearing of the leaves (di Franco et al., 1979; Russo et al., 1979). Recently, an icosahedral virus-like particle has been detected in thin sections of 'Mexicana' mesophyll cells (G. Minas, 1980, unpublished data). The latter reports suggest that PNVA and PPSA may have similar causations.

This study was undertaken to determine the behaviour of these agents in meristem culture and to study their potential singly, and together, as beneficial infectious agents in other commercially important ivy-leaf and zonal pelargoniums. Here the term "beneficial infection" infers that these agents do not prevent the commercial exploitation of the affected cultivars.

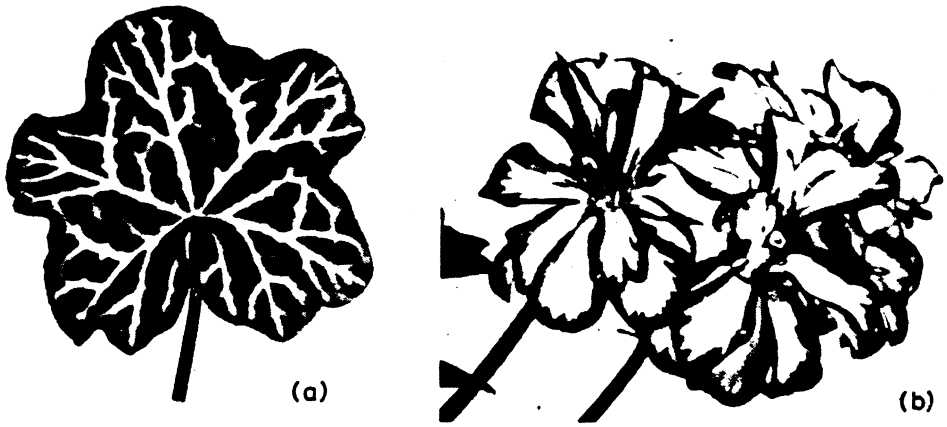

Fig. 1. (a) Leaf of 'Crocodile' showing vein clearing. (b) Flower of 'Mexicana' showing petal streak.

MATERIALS AND METHODS

Plant material. — *Pelargonium peltatum* 'Crocodile' (syn. 'Sussex Lace') and 'Mexicana' (syn. 'Roulette') were the sources of PNVA and PPSA, respectively. The following cultivars were used for graft transmission and other tests:

Pelargonium × *hortorum* (zonal "geraniums") 'Brook's Purple', 'Cardinal', 'Empress', 'Genie', 'Hakaart', 'Irene Red-Purple', 'Kleine Liebling', 'Layton White', 'Master Crampel', 'Maxim Kovalevski', 'Orange Flyn', 'Orange Ricard', 'Paul Crampel', 'Penny', 'Prince of Denmark', 'Pygmalion', 'Skelly's Pride', 'Sophie Koniger'.

Pelargonium peltatum (ivy-leaf "geraniums") 'Abel Carriere', 'Ailsa Garland', 'Fin d'Amour', 'Fire Bird', 'Galilee', 'La France', 'Mexican Beauty', 'Mrs. Warligton', 'Old Lady', 'Rigi', 'Super Rose', 'Yale'.

P. × *hortorum* × *P. peltatum* (semi-ivy-leaf "geranium") 'Kewense', 'Schöne Grentchen'.

Plant cultivation — Plants were grown throughout the year in a glasshouse

with a minimum temperature of 15°C in Irish Peat Moss supplemented with Bio P Base fertilizer (Pan Britannica Industries, Herts, U.K.). Flowering was stimulated out-of-season by placing the plants in 10°C-chambers in the glasshouse with a 16-h photoperiod provided by fluorescent lamps.

Meristem culture. — Shoot tips were excised and the apical domes cultured on the medium of Hamdorf (1976), with slight modification as described previously (Cassells et al., 1980) (see Table I).

Adventitious shoots (approximately 2 cm in height) from these cultures were placed on rooting medium (Cassells et al., 1980) (see Table I) and following root formation, plantlets were established on heated benches (15°C) and transferred to insect-proof cages, as appropriate, for virus testing.

TABLE I

Tissue culture media used for meristem proliferation and rooting of progeny shoots; pH adjusted to 5.8

Constituents	mg/l	g/l
Meristem medium		
MS[1] basal medium without growth substances		4.71
Ammonium nitrate		0.825
Sodium dihydrogen phosphate		0.15
Casein hydrolysate		1.0
Sucrose		30.0
Indoleacetic acid	2.0	
Gibberellic acid (GA_3)	1.0	
Kinetin	4.0	
Adenine sulphate	50.0	
Meso-inositol	100.0	
Agar		6.0
Rooting-medium		
MS basal medium without growth substances		2.36
Sucrose		15.0
Kinetin	0.01	
Indoleacetic acid	0.1	
Agar		6.0

[1] Murashige and Skoog (1962).

Disease transmission and testing. — Geranium shoots with PPSA or PNVA symptoms, or symptomless plants from tissue culture, as appropriate, were used as scions. The stocks were symptomless rooted cuttings of the cultivars listed above (see text also) or symptomless *P* × *hortorum* seedlings.

A clean oblique downward cut was made in the stem of the stock and the detached scion inserted so that the cambial regions were aligned as

completely as possible. The graft was bound with self-adhesive rubber tape (Stericrepe;Beacon and Janis Ltd., London). Two leaves were left on the scion and half of the leaves of the stock were removed, along with the apical bud, to stimulate the growth of axillary buds. Grafted plants were placed in a mist propagator for 4—6 weeks and when a good graft union was established they were returned to normal glasshouse conditions. Plants were observed for up to 18 months for symptoms.

RESULTS

The initial graft transmission studies were between 'Crocodile' which has a pale rose-pink flower (PNVA-infected) and 12 other ivy-leaf cultivars. There were 10 replicates in all cases, which were almost all successful and resulted in positive transmissions. In some cases, not confined to specific cultivars, serious leaf distortion was observed in the first-formed new leaves on the stock. However, foliar symptoms stabilized in all cultivars and resembled those of 'Crocodile'.

In a further series of graft-transmission studies (10 replicates of each as above) 'Crocodile' (PNVA) was grafted to 18 zonal geraniums and 2 zonal—ivy-leaf hybrids. In almost all cases, graft transmission was observed. The net vein symptoms first appeared in the newly formed leaves on the stock adjacent to the scion and were severe and greatly reduced leaf area (Fig. 2). Then, depending on the growth of the stock, the symptoms tended to be less pronounced or to disappear. Symptoms tended to reappear in early spring and late summer.

Fig. 2. Graft transmission of PNVA from 'Crocodile' to zonal 'Paul Crampel'. Net vein symptoms in the young leaves above the graft union.

Fig. 3. Symptoms of PPSA in zonal 'Paul Crampel'.

PPSA was successfully graft-transmitted from 'Mexicana' to 12 ivy-leaf cultivars and to 2 ivy-leaf × zonal hybrids. In all cases, petal streak symptoms of the same appearance as those in 'Mexicana' were observed. In some single-flower cultivars, curling of the petals was observed.

Graft-transmission of PPSA to zonal geraniums resulted in serious petal curling and distortion in most cultivars. In others, the symptoms were less pronounced, or absent from the face of the petal but appeared as clearing of pigment from the petal veins when viewed from the back (Fig. 3). The symptoms produced in the zonal ivy-leaf hybrid 'Schöne Grentchen' (vein clearing on both petal surfaces) were the most attractive in this series of transmissions.

In the final studies on graft-transmission, PNVA and PPSA were the subject of double graft transmission to a series of ivy-leaf cultivars. The results show that double transmission was achieved in all cases and there was no apparent interaction between PPSA and PNVA in the doubly infected plants.

Meristem culture is now an established commercial procedure for the propagation of commercial pelargoniums. PNVA has previously been shown to be eliminated in meristem culture of 'Crocodile' (Cassells et al., 1980). Shoot-tip cultures were set up here to determine whether PPSA and PNVA were transmissible. Meristem culture of 'Crocodile' was repeated. The data (Table II) show that progeny plants derived via meristem culture from both PPSA- and PNVA-infected cultivars were symptomless from observations over 12 months in the glasshouse. Furthermore, PNVA or PPSA symptoms were not produced in symptomless 'Paul Crampel' seedlings to which the meristem cultured plants were grafted.

TABLE II

Propagation of plants from shoot tips of PPSA or PNVA affected geranium cultivars

Geranium cultivar	Affected by	No. of cultured meristems	No. of established meristems	No. of adventitious shoots in 8 months since cultures were started	No. of plants potted	No. of plants affected, observation of leaves and flowers for 1-year growing-period
Paul Crampel	PPSA	100	15	93	21	0
Mexicana	PPSA	120	12	95	36	0
Rigi	PPSA	80	7	75	21	0
La France	PPSA	80	15	92	31	0
Crocodile	PNVA	120	18	123	48	0
Rigi	PNVA	80	6	64	22	0
La France	PNVA	80	5	71	18	0

DISCUSSION

As a preface to this discussion, it is important to mention that in the course of these studies over 3 years, neither PNVA or PPSA were observed to spread naturally in the glasshouse or field. Indeed, many attempts to sap-transmit, or to achieve vector transmission, were unsuccessful (K.W. Bailiss, G. Minas and A.C. Cassells, 1980, unpublished data). Consequently, there appears to be little risk of uncontrolled spread of these agents, possibly both viral, within the commercial nursery.

Both PNVA and PPSA are readily graft-transmissible to all ivy-leaf and zonal cultivars tested. Neither, however, produce attractive, or in the case of PNVA, stable symptoms in zonal cultivars. Consequently, they have little commercial potential in zonal cultivars.

Both PNVA and PPSA, singly or together, have considerable commercial potential in ivy-leaf cultivars. PNVA transforms ivy-leaf geraniums into fancy leaf cultivars, which may encourage earlier purchase, i.e. as foliage rather than flowering plants. Furthermore, while 'Crocodile', the source of PNVA has a relatively pale flower, the presence of PNVA in e.g. 'Abel Carriere', which has a strong flower colour (orchid purple), makes an attractive plant (Fig. 4a). The potential for PPSA-transformed cultivars may be less than for those altered by PNVA, because much of the attraction of PPSA-affected flowers lies in the contrast between the pigment and white (pale) area on the petal. Where the petal pigment is originally pale, this effect is reduced. However, with strong flower colours, e.g. 'Yale' which has a red flower, the effect is attractive (see Fig. 4b).

The combination of PPSA and PNVA in doubly infected cultivars results in the production of a third series of beneficially infected cultivars. Those with a strong flower colour-contrast also have commercial potential (Fig. 4c). 'Crocodile' and 'Mexicana', sources of PNVA and PPSA, respectively, are

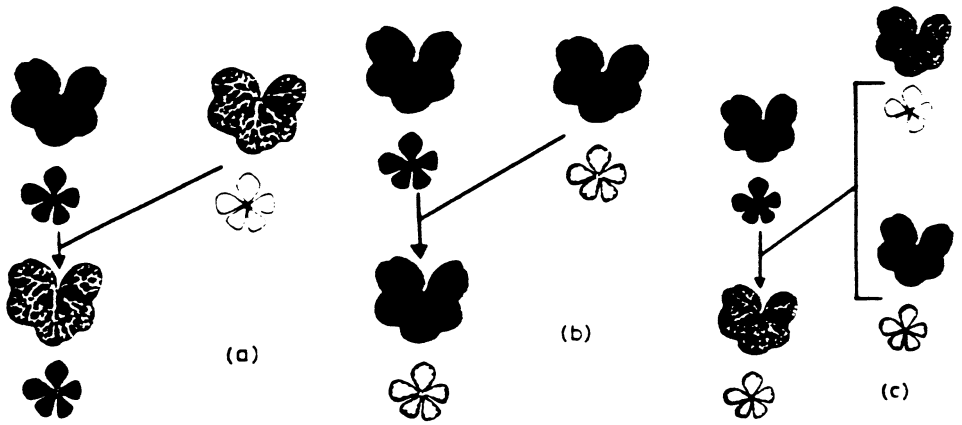

Fig. 4. (a) Schematic representation of the transfer of PNVA from 'Crocodile' which has a pale flower to a cultivar with a strong flower colour to produce a "new" cultivar. (b) Schematic representation of the transfer of PPSA from 'Mexicana' to another cultivar, e.g. 'Abel Carriere', with a strong flower colour, to produce a "new" cultivar. (c) Schematic representation of the transfer of both PNVA and PPSA to another cultivar to produce a "new" doubly-infected cultivar.

only two of many pelargonium cultivars with abnormal pigmentation. There are numerous similar examples of abnormal pigmentation induced by beneficial infections affecting other ornamental crops, e.g. abutilon and tulip (Gibbs and Harrison, 1976).

Our studies emphasize the problems facing the micro-propagator who introduces meristem culture for such cultivars, and poses the problem of whether such beneficial infections can or should be exploited commercially where there is the possibility of disease spread.

The results presented here pose a dilemma for the commercial pelargonium propagator who uses meristem culture, or is proposing to introduce it, for meristem culture apparently cannot be used to propagate these beneficially infected cultivars. Either the micro-propagator must use explant culture (Cassells et al., 1980) or re-inoculate the plants with the beneficial agent(s). Explant culture may not be efficient in that, for example, regeneration may be limited (Cassells, 1979). The causal agent(s) of PNVA and PPSA have not been isolated, purified and fully characterized, thus re-inoculation is a problem.

Stone and Hollings (1973) isolated a flower-break virus (PFBV) from *P* × *hortorum* 'Irene' and 'Paul Crampel'. Unlike PPSA reported here, PFBV induced flower colour break in these cultivars throughout the year. More recently, di Franco et al. (1979) and Russo et al. (1979) identified a vein-clearing virus in *P. peltatum* (PVCV) resembling PNVA described in this work. However, the symptoms of PVCV, shown to be a sap-transmissible rhabdovirus, appear to differ from those of PNVA. The present authors have failed to detect rhabdovirus-like particles in the cells of PNVA-affected

pelargoniums, nor have they been able to sap-transmit PNVA. Further studies are required to elucidate the relationship of PNVA and PPSA to PVCV and PFBV, respectively.

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