Investigations on Viruses of Pinks *(Dianthus Sp.)* in the United Kingdom

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Abstract

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Viral diseases were detected in eight varieties of commercial glasshouse pinks *(Dianthus sp.)* in the Exeter Area. The diseases were so prevalent and all commercial stocks were found infected by 3 or 4 viruses, VIZ. Carnation etched ring virus (CERV), carnation latent virus (Car LV.). Carnation ring spot virus (CRSV) and carnation vein mottle virus (Car VMV). The identity of these viruses was established by a combination of host range and symptomatology, physical characteristics and serology. The aphids *Myzus certus* (Walker) and *Neomyzus circumflexus* (Buckton) were recorded as new vectors for CERV, Car LV and Car VMV. New susceptible plant species for the 4 viruses were found.

Key words: glass house pinks, viruses, England.

Introduction

True «Pinks», members of the Caryophyllaceae family, have been derived from *Dianthus plumaris* L. which was introduced to Britain from Eastern Europe in about 1629. Since then it has given rise to many varities (Chitteden, 1956), show pinks being developed from strains of allwoodii hybrid pinks (Simcock, 1973). As pinks and carnations are closely related species and propagated in a similar way they would be expected to be susceptible to similar viral diseases.

Twelve viruses have been characterised from British carnation beds (9). But only five are considered of importance in this country (5). These are: carnation etched ring virus (CERV), carnation latent virus (Car LV), carnation mottle virus (Car MV), carnation ring spot virus (CRSV) and carnation vein mottle virus (Car VMV). The present work was undertaken to investigate the occurrence of these viruses, and carnation necrotic fleck virus (CNFV) whose presence was suspected on the basis of symptomatology, in commercially grown glasshouse show pinks in the Exeter area.

Materials and Methods

Collection of samples: Pinks plant samples were obtained mainly from a major supplier of cuttings at Haytor nursery and other cutflower growers in the Exeter area. The source cultivars were: Christopher, Dennis, Diane, Doris, D 26/16, Haytor, Joy and Ruby.

Test plants: The commonly used indicator plants for carnation viruses, namely *Chenopodium amaranticolor* Coste and Reyn, *Chenopodium quinoa* Wild, *Dianthus barbatus* cv. Scarlet Beauty, *Comphrena globosa* L. and *Silene armeria* L. were tried in present work. Plants were grown in soil compost inside an insect proof glasshouse compartment maintained at 15 - 20°C.

Virus isolates: Isolates of 4 detectable viruses were obtained from pinks by inoculation to differential hosts including *C.amaranticolor, C. quinoa, D. barbatus* cv. Scarlet Beauty, *G. globosa* and *S. armeria.* The isolates were passed through single local lesions in *S.armeria*(for CERV), *C. quinoa* (for Car LV and CRSV) and D. barbatus CV. Scarlet Beauty (for Car VMV) and further inoculations near dilution endpoint to appropriate hosts plants in which they were maintained and used throughout the present work, thus CERV and Car LV were inoculated and maintained separately in *S. vaccaris* CV. Pink Beauty, CRSV in *G. globosa* and Car VMV in *D. barbatus* CV. Scarlet Beauty.

Methods of Testing:

Sap inoculation: Sap inoculation was performed as described by Noordam (11).

Aphid transmission: Virus-free *Myzuz persicae* (Sulzer), *M. certus* (Walker), *Neomyzus circumflexus* (Buckton), *Aulacorthum solani* and *Macrosiphum euphorbiae* were fasted for 1hr. *Aphids* were fed for 5 - 10 min on virus-infected sources before their transfer, in groups of 10, for inoculation feeding to rooted cuttings of healthy *D. allwoodii* pinks for 15 - 20 min.

Serology: Gel-diffusion tests were done on microscope slides using 10 gm/1 Ion Agar No. 2 (Oxoid) and 8.5g/l NaCl in 0.01M phosphate buffer at pH 7.0. Wells in the agar plate were 6 mm in diameter and 3 mm apart. The plates were read 24 hours later at c. 20°C.

Electron microscopy: Virus extracts were examined after negative staining (2 % phosphotungstate) on |formvar-coated copper grids in an SEI EM 801 electron microscope.

Light microscopy: Infected pinks plants were examined for inclusion bodies as described by Robb (12).

Physical properties: The 3 properties, thermal inactivation point, longevity in *vitro* and dilution-end point were determined for each virus by testing sap extracted from source plants.

Table 1:. Incidence of detectable viruses in commercial pinks.

جدول 1. انتشار الفيروسات التي يمكن الكشف عنها في القرنفل التجاري.

		الفيروسات Viruses							
	Number of samples tested	CERV		Car LV		CRSV		Car VMV	
Variety		No. of infected plants	percent infection	No. of infected plants	Percent infection	No. of infected plants	Percent infection	No. of infected plants	Percent infection
الأصناف	عدد العينات المختبرة	عدد النباتات المصابة	% للاصابة	عدد النباتات المصابة	% للاصابة	عدد النباتات المصابة	% للاصابة	عدد النباتات المصابة	% للاصابة
Christopher	30	30	100	30	100	0	0	30	100
Dennis	46	46	100	46	100	0	0	46	100
Diane	81	81	100	81	100	0	0	81	100
Doris	130	130	100	130	100	0	0	130	100
D 26/16	11	11	100	11	100	0	0	11	100
Haytor	79	79	100	79	100	0	0	79	100
Joy	77	77	100	77	100	77	100	77	100
Ruby	11	11	100	11	100	0	0	11	100

Table 2. Aphid transmission tests with viruses isolated from pinks to healthy *D.allwoodii* seedlings.

جدول 2. اختبارات النقل بالمنّ للفيروسات المعزولة من القرنفل على بادرات *D. allwoodii*

	CERV		CarLV		Car VMV		CRSV	
Aphid species	No infected / No inoculated	% trans-mission	No infected / No inoculated	% trans-mission	No infected / No inoculated	% trans-mission	No infected / No inoculated	% trans-mission
أنواع المن	عدد المصاب / عدد الملقح	% للنقل	عدد المصاب / عدد الملقح	% للنقل	عدد المصاب / عدد الملقح	% للنقل	عدد المصاب / عدد الملقح	% للنقل
Myzus certus	24/49	49.0	32/45	71.1	27/48	56.25	0/36	0
M. persicae	24/50	48.0	31/45	68.9	28/48	58.3	0/36	0
Neomyzus circumflexus	15/46	32.6	24/47	51.1	19/48	39.6	0/36	0
Aulacorthum solani	0/32	0	0/30	0	0/32	0	0/30	0
Macrosiphum euphorbiae	0/33	0	0/30	0	0/33	0	0/30	0

Table 3. Physical properties of the four pinks viruses investigated in this study.

جدول 3. الخصائص الفيزيائية لأربع فيروسات تصيب القرنفل.

Virus الفيروس	Thermal inactivation درجة الحرارة المثبطة	Dilution end point نقطة التخفيف النهائية	Longevity *in vitro* at 20°C التعمير خارج العائل عند درجة حرارة 20°م	Source plant المصدر النباتي
CERV	75 - 80°C	10^{-3} - 10^{-4}	96 - 120 hr	*S. vaccaria* Cv. Pink Beauty
Car LV	60 - 65°C	10^{-3} - 10^{-4}	48 - 72 hr	*S. vaccaria* Cv. Pink Beauty.
CRSV	85 - 90°C	10^{-5} - 10^{-6}	25 - 30 day	*G. globosa*
Car VMV	55 - 60°C	10^{-3} - 10^{-4}	96 - 120 hr	*D. barbatus* Cv. Scarlet Beauty

Table 4. Host range and symptomatology of CERV, Car LV, CRSV, and Car VMV.

جدول 4. المجال العائلي والأعراض لفيروسات CERV، Car LV، CRSV، Car VMV

الأنواع النباتية Plant species	Virus الفيروسات			
	CERV	Car LV	CRSV	Car VMV
Amaranthaceae:				
Amaranthus caudatus	–	–	–	+ L
Gomphrens globosa			+ LS	–
Caryophyllaceae:				
Dianthus allwoodii	+ LS	+ SS	+ LS	+ S
D. barbatus Cv. Scarlet Beauty	+ LS	+ SS	+ LS	+ S
Gypsophila elegans CV. Covent Garden white	+ LS	+ SS	+ LS	+ S
Saponaris officinalis	–	+	+ SS	–
S. vaccaria Cv. Pink Beauty	+ LS	+ SS	+ LS	+ S
S. vaccaria Cv. White Beauty	+ LS	+ SS	+ LS	+ S
Silene alba	+ SS	+ SS	+ S	+ SS
S. armeria	+ LS	+ SS	+ LS	+ S
S. behen	+ LS	+ SS	+ S	+ SS
S. clorata	+ LS	+ SS	+ LS	+ SS
S. coeliroses	+ S	+ SS	+ LS	+ SS
S. compacta	+ S	NT	NT	–
S. conica	–	+ SS	+ SS	+ SS
S. conoidea	+ LS	+ SS	+ LS	+ SS
S. cretica	+ S	–	+ LS	–
S. dichotoma	+ S	+ SS	+ LS	+ SS
S. dioica	+ SS	+ SS	+ S	+ SS
S. friwaldskyane	–	–	NT	NT
S. gallica	+ S	+ SS	+ SS	NT
S. gigantea	+ SS	+ SS	+ S	NT
S. italica	–	–	–	NT
S. mellifera	+ S	NT	–	NT
S. muscipula	NT	+ SS	NT	NT
S. noctiflora	+ S	+ SS	+ SS	+ SS
S. nocturna	+ SS	+ SS	+ S	+ SS
S. nuianus	–	NT	NT	
S. otites	–	+ SS	+ SS	NT
S. pendula	+ LS	+ SS	+ LS	+ S
S. viridiflora	+ SS	VT	+ S	NT
S. vulgaris	–	–	–	–
S. latifolia	+ S	NT	NT	NT
Stellaria media	+ SS	+ SS	+ SS	+ SS
Chenopodiaceae:				
Beta vulgaris Cv. Crimson Globe	–	+ S	NT	+ SS
Chenopodium album	–	–	+ L	–
C. amaranticolor Coste and Reyn	–	–	+ L	+ L
C. quinoa	–	+ LS	+ L	+ L
C. murale	NT	–	NT	–
Spinacea oleracea Cv. Round or Summer	NT	–	NT	–
Compositae:				
Helianthus annus	–	–	–	–
Lactuca sativa Cv. Lobjoit	NT	–	SS	–
Cucurbitaceae:				
Cucumis sativus Cv. Telegraph	–	–	+ L	–

الأنواع النباتية Plant species	الفيروسات			
	CERV	Car LV	CRSV	Car VMV
Cruciferae:				
Brassica oleracea Cv. Capitata	NT	NT	NT	–
B. pekinensis Cv. Petsai	NT	–	–	–
Raphanus sativus Cv. cherry Belle	NT	–	–	–
Leguminosae:				
Phaseolus coccineus Cv. Scarlet Runner	NT	–	–	–
P. vulgaris Cv. Masterpiece	–	–	+ LS	–
Vigna sinensis Cv. Blackeye	–	–	+ LS	–
Solanaceae:				
Nicotiana bigeloveii	NT	NT	+ LS	–
N. clevelendii	–	+ S	+ LS	–
N. glutinosa	–	–	+ L	–
N. rustica	–	–	+ L	–
N. tabacum Cv. White Burley	–	–	+ L	–
N. tabacum Cv. Samsum NN	–	–	+ L	–
Petunia hybrida Cv. Bedding Mixed	–	–	+ SS	–
Physalis floridana	–	–	+ S	+
Plantaginaceae:				
Plantago indica	NT	–	–	+ L
P. lagopus	–	–	NT	–
P. lanceolata	–	–	+ SS	+ L
Portulaceae:				
Claytonia perfoliata	–	NT	NT	NT
Violaceae:				
Viola tricolor	–	–	NT	–

Abbreviations used: (+) infected, (−) not infected, (L) Local lesion, (S) systemic infection, (SS) symptomless systemic infection, (NT) not tested.

المختصرات المستخدمة: (+) مصاب، (−) غير مصاب، (L) بقع موضعية، (S) إصابة جهازية، (SS) إصابة جهازية بدون أعراض، (NT) لم يتم اختباره.

Host range and symptomatology: A number of plant species were tested by sap inoculation for susceptibility to the isolated viruses.

Results

Sap inoculation: Results of infectivity tests indicated the following reactions:

C. amaranticoler: yellow-green to semi-necrotic local lesions in 7 – 12 days from inoculation. Those symptoms were not often consistent.

C. quinoa: Diffuse chlorosis and chlorotic spots on inoculated leaves 7 – 11 days from inoculation, followed in 2 – 3 weeks by systemic interveinal yellowish spotting, puckering and leaf dwarfing particularly those on axillary shoots.

D. barbatus cv. Scarlet Beauty: Systemic vein clearing, chlorotic spotting and leaf mottling in 7 – 13 days.

G. globosa: Local necrotic concentric rings and ring spots in 4 – 5 days, followed by systemic mottling, flecking and malformation of young leaves.

S. armeria: Systemic necrotic lines, ring pattern and blotches developed on the leaves 2 – 3 weeks from inoculation.

On the bases of those results four viruses (CERV, Car LV, CRSV and Car VMV) were suspected to infect commercial pinks.

The overal results of the sampling of commercial pinks (Table 1) showed clearly the high incidence of CERV, Car LV and Car VMV in the 8 varieties tested from all sources sampled. Moreover, results showed that only one variety, joy, was also infected with CRSV.

Aphid transmission: Table 2 indicated that CERV, Car LV and Car VMV were transmitted in the non-persistent manner from source plants to *D. allwoodii* seedling pinks by the aphids *M. persicae, M. certus* and *N. circumflexus.* Repeated attempts to transmit those viruses by *A. solani* or *M. euphorbiae* were unsuccessful. Survey indicated that *M. certus* was more frequently infesting the pinks crop.

Serology: Clarified sap extracted from any of the 8 varieties produced strong reactions against the following antisera: Car LV (1/40 – 1/1280), Car VMV (1 /12 – 1/384); faint reaction

against CERV (1/4 - 1/32) and failed to react with CRSV (1/16 - 1/512) with the exception of Joy. Healthy control preparations failed to react with any of these antisera at the dilution used. No serological evidence for the presence of Car MV (1/15 - 1/280) or CNFV (1/2 - 1/32) was obtained.

Electron microscopy: Negatively-stained specimens readily revealed several flexuous filamentous particles (c. 790 × 12 nm) and also straight to slightly curved rods (c. 654 × 12 nm) typical to those of Car VMV and Car LV, respectively. A few spherical particles in the range of CRSV (c. 30 nm) and CERV (c. 50 nm) were discernible.

Light microscopy: A prominent round or slightly elliptical refringent body was visible. Appearance and size were very similar to those described for CERV by Fujisawa *et al.* (2).

Host range and symptomatology: Results are summarised in Table 4. CERV infected 23 of 50 species from one (Caryophyllaceae) of 8 families; Car LV infected 25 of 52 species from 4 of 11 families; CRSV infected 34 of 48 species from 7 of 9 families and Car VMV infected 23 of 50 species from 4 of 10 families. New susceptible species especially in Caryophyllaceae were identified for all 4 viruses.

Physical properties: These are summarized in Table 3.

Discussion

Although pinks are becoming an important ornamental crop in commercial glasshouses in South West England, it has been shown here that they are subject to serious attack by viruses. Of these, CERV, Car LV, CRSV and Car VMV seem to be the main viruses infecting this crop. The identity of the 4 viruses was established on the bases of symptomatology, physical properties, serology, particle morphology and/or inclusion bodies.

The differential reactions manifested on *S. armeria, G. globosa, C. quinoa* and *D. barbatus* were typical to those described for CERV (3), Car LV (6), CRSV (7) and Car VMV (9), respectively. Those above-mentioned hosts proved reliable indicators for the diagonsis of those 4 viruses throughout the course of this study.

Unlike the Glasshouse Crop Research Institute isolates of Car VMV (4) the pinks isolates of this study infected *C. amaranticolor* with some difficulty, induced local but not systemic reaction on *C. quinoa* and failed to infect *G. globosa.* The pinks isolates of Car LV failed to infect *C. amaranticolor,* reported susceptible by Wetter (14) with an isolate from carnation.

The host range study, strongly biased towards Caryophyllaceae species, suggested that most members of this family were susceptible, in many cases to all 4 viruses and therefore could be potential reservoirs and sources of infection.

The aphids *M. certus* and *Neomyzus circumflexus* were shown here to be new vectors of CERV, Car LV and Car VMV. *M. certus* is especially important; its frequent occurrence, high population density makes it more dangerous than either *M. persicae,* a known vector (9, 10, 14) or *N. circumflexus.* The latter was the least prevalent and the least efficient vector of the three.

The identified 4 viruses appeared to be widespread in commercial pinks stock and occured in mixed infections. Investigations here-with indicated that every sample examined from each of the 8 varieties of pinks was mix-infected with at least 3 viruses (Table 1). The fact, as was learnt later, that those samples collected from parents mainly supplied by one major grower sheds much light on the results obtained. The present study raises an interesting question regarding (a) the absence of Car MV, the most infectious and widespread virus in carnation culture (8) and (b) the high incidence of Car LV and Car VMV in pinks in contrast with their rare occurrence in carnation (5). This suggests that the virus status in pinks is different from that in carnations.

الملخص

محمد عبد الماجد، عبد القادر وشيلا روب. دراسة حول الأمراض الفيروسية التي تصيب زهرة القرنفل في المملكة المتحدة (إنكلترا). مجلة وقاية النبات العربية 8 (2): 121-126.

كُشفت أمراض فيروسية في ستة أصناف تجارية من القرنفل (*Dianthus* sp.) المزروع في منطقة Exeter. وقد كانت الأمراض منتشرة بشدّة، وتبيّن أن جميع الأصول التجارية مصابة بثلاثة أو أربعة فيروسات (CERV، Car LV، CRSU، Car VMV). وقد تمّ التحقق من هويّة هذه الفيروسات بوساطة المجال العائلي، والأعراض، والصفات الفيزيائية والمصلية/ السيرولوجية. وقد تم تسجيل نوعان جديدان من المنّ *Neomyzus circumflexus* (Buckton.), *Myzus certus* (Walker) كناقل لثلاثة من هذه الأمراض. كما وجدت أنواع نباتية جديدة قابلة للاصابة بالفيروسات الأربعة.

كلمات مفتاحية: قرنفل، فيروسات، بريطانيا.

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