Characterization and Classification of *Erwinia chrysanthemi* Strains from Several Hosts in The Netherlands

Jaap D. Janse and M. A. Ruissen

Bacteriologist, Department of Bacteriology, Plant Protection Service, Geertjesweg 15, 6700 HC Wageningen, The Netherlands; and bacteriologist, Department of Phytopathology, Agricultural University, Binnenhaven 9, 6709 PD Wageningen, The Netherlands.
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ABSTRACT

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Forty-one strains of the bacterium *Erwinia chrysanthemi* were characterized using physiological, biochemical, serological, and pathogenicity tests. Strains from The Netherlands originated from *Aechmea fasciata*, *Aglaonema*, *Cichorium intybus*, *Dieffenbachia*, *Kalanchoë blossfeldiana*, *Philodendron erubescens*, *Scindapsus pictus*, and *Solanum tuberosum*. Reference strains were from *Chrysanthemum morifolium*, *Dianthus caryophyllus*, *Dieffenbachia*, *Philodendron*, *S. tuberosum*, and *Zea mays*. On the basis of growth on eight different carbon sources, growth at 39 C, and degradation of arginine, we found that four of the seven biovars of *E. chrysanthemi* occurred among strains from The Netherlands (biovars 2, 3, 5, and 7). Most potato strains and *Kalanchoë* strains belong to biovar7; the other potato strains and one from *C. intybus* belong to biovar 5. *Dieffenbachia* strains belong to biovar 2, and strains from *Aechmea*, *Aglaonema*, *Philodendron*, and *Scindapsus* belong to biovar 3. Using seven antisera against three somatic (O) serogroups and three flagellar (H) serotypes of *E. chrysanthemi*, strains were classified using indirect immunofluorescence. Only serogroup 1 was found among strains from The Netherlands. The most common combination was serogroup 1-flagella type 1 (O1:H1). Strains from *Aglaonema*, *Dieffenbachia*, *Philodendron*, and *Scindapsus* showed unknown flagella types (O1:H?). Some reference strains from corn and chrysanthemum belong to an unknown serogroup. There was no correlation between serogroup-flagella type and biovar or original host. *E. chrysanthemi* strains were tested for pathogenicity on *K. blossfeldiana* 'Calypso,' *P. erubescens* 'Emerald King,' *S. tuberosum* 'Bintje,' and *Z. mays* 'LG 11.' All strains were pathogenic for corn and potato. *Kalanchoë*, cichory, and potato (KCP) strains were not pathogenic on *Philodendron*; the other strains (from greenhouse crops and corn) were pathogenic. On *Kalanchoë* only the KCP strains caused infections. Moreover, the KCP strains did not grow at 39 C and showed a much weaker pectolytic activity at 37 C than greenhouse and corn strains. KCP strains of *E. chrysanthemi* apparently form a separate group, adapted to crops grown under temperate conditions.

The need to standardize classification techniques is discussed.

Erwinia chrysanthemi Burkholder et al is a gram-negative, plant pathogenic, pectolytic bacterium belonging to the enterobacteriaceae family. It is phenotypically closely related to the pectolytic *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* (16) and to a lesser extent to the genera *Klebsiella* and *Serratia* (3). A distinction between the above-mentioned subspecies of *Erwinia* can be made by biochemical and physiological tests (35) and by difference in pectolytic activity at different temperatures (34).

E. chrysanthemi has a high optimum growth temperature (35–37 C). Because it occurs in a number of greenhouse ornamentals, the bacterium has a worldwide distribution (4). The host range of *E. chrysanthemi* is very large and comprises not only ornamentals (10,11) but also food crops such as *Allium* spp. (11), *Cichorium intybus* (this study, 40), *Daucus carota* (44), *Ipomea batatas* (41), *Oryza sativa* (17), *Solanum tuberosum* (6), and *Zea mays* (39).

Strains from different host plants differ in host range and pathogenic and phenotypic properties (10,11).

The first biochemical subdivision of strains of *E. chrysanthemi* was made by Lelliott (28), who recognized four subspecies. These subspecies were named by Young et al (48): *E. chrysanthemi* pv. *chrysanthemi*, pv. *dieffenbachiae*, pv. *parthenii*, and pv. *zeae*. Dickey (10) divided 322 strains into five biochemical groups. Four of these groups were named after the above-mentioned pathovars and the fifth group was named pv. *dianthicola*. Dickey and Victoria (12) recognized *E. chrysanthemi* strains from *Musa paradisiaca* as pv. *paradisiaca*. The most recent biochemical subdivision, developed by Samson and Nassan-Agha (38) and Samson et al (40), is based on tests with the six pathotypes (type-strains of the above-mentioned pathovars and isolate CFBP 2015 from potato). In total, seven groups, named biovar 1 to 7, were distinguished.

Serologically, strains of *E. chrysanthemi* could be subdivided in four serogroups on the basis of somatic (O) antigens (13,37). Later Samson et al (40) distinguished at least three O serogroups and three different flagellar (H) antigenic types.

In The Netherlands, *E. chrysanthemi* has been found on a number of ornamentals, including *Aechmea*, *Dieffenbachia*, *Kalanchoë*, *Philodendron*, and *Scindapsus* (21–23), and on potato (*S. tuberosum*). The bacterium has not been found on corn (*Z. mays*). It was not known to which biovar-pathovar or serogroup-flagella type *E. chrysanthemi* strains from The Netherlands belonged. Furthermore, it was not known if strains from certain hosts would be a threat for other hosts. In this paper we describe the characterization and classification of 31 *E. chrysanthemi* strains from The Netherlands from different hosts, using physiological, biochemical, serological, and pathogenicity tests. We also include 10 reference strains from different hosts and origin and one isolate from *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora*.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the 43 bacterial strains used, with their identity and origin. All strains were maintained on nutrient agar (NA, Difco, Detroit, MI) with 0.1%, w/v, D(+)glucose, and all strains were lyophilized.

Morphological properties. Colony characteristics were determined after 2 days incubation at 27 C on yeast-peptone-glucose agar (YPGA), containing (w/v) 0.5% yeast extract, 1% peptone, 0.5% D(+)glucose, 1.5% agar. Size of the bacterial cells and reaction in the Gram stain were determined using 24-hr NA cultures. Motility was examined in a drop of nutrient broth (NB, Difco) in dark field microscopy, using 24-hr NA cultures.

Physiological and biochemical properties. Growth at 39 C was determined after 24-hr growth of NB cultures in a water bath. Salt tolerance was checked after 48-hr growth in NB with 5%, w/v, NaCl.

For oxidase activity, Kovács' (26) method was applied. To detect catalase activity, a loopful bacteria of a 24-hr NA culture was smeared into a drop of 10% H_2O_2. H_2S production was determined using Skermans' (42) method. Gelatin hydrolysis, nitrate reduction, and anaerobic breakdown of L(+)arginine were detected by the methods of Lelliott et al (29). Pectolytic activity was determined on a pectate medium according to Perombelon (33) or on the crystal violet pectate medium (CVP) of Cuppels and Kelman (7). Cellulolytic activity was checked with a viscosity meter after 4 days growth in a medium containing (w/v) 0.5% Na-taurocholate; 0.1% $NH_4H_2PO_4$; 0.02% $MgSO_4 \cdot 7H_2O$; 0.02% KCl; 0.005% bromothymol blue; 0.3% $CaCO_3$; 8% carboxymethyl-cellulose (Sigma Chemical Co., St. Louis, MO). To test for indol production, the method of Kovács (1) was used. Glucose metabolism was detected in Hugh and Leifson's (20) medium. Production of reducing substances of K-gluconate and sucrose was detected by the methods described by Lelliott and Dickey (30).

Alkali production from organic acids was detected using the method of Collins and Lyne, modified by Webb (46), on a medium containing (w/v) 0.1% $MgSO_4 \cdot 7H_2O$; 0.1% NaCl; 0.08% yeast extract; 0.1% $(NH_4)_2HPO_4$; 0.05% KH_2PO_4; 0.03% phenol red; 1.2% agar; and 0.5% organic acid, pH 6.8, with NaOH. Growth on carbon sources other than organic acids was recorded on the minimal medium (MM) of Ayers et al (2) using replica plating. Carbon sources (1%, w/v) were included in the media and sterilized 10 min at 110 C. Gallate, citrate, ethanol, and γ-aminobutyrate were filter sterilized (0.2 μm millipore) and added to MM (1%, w/v). All carbon sources were tested in duplicate.

Serological properties. Table 2 lists the origin and homologous

TABLE 1. Origin and identity of strains used in this study

Strain	Host	Country
Erwinia chrysanthemi		
PD[a] 382	*Aechmea fasciata* (Ldl.) Bak.	Netherlands
PD 430	*Aglaonema* 'Silver Queen'	Netherlands
82/21	*Cichorium intybus* L. 'Zoom'	Netherlands
NCPPB[b] 402	*Chrysanthemum morifolium* Ramat. (co-type)	United States
NCPPB 2339	*Chrysanthemum morifolium* Ramat.	United Kingdom
CFBP[c] 1200	*Dianthus caryophyllus* (= NCPPB 453, pathotype)	United Kingdom
NCPPB 2976	*Dieffenbachia* sp. (pathotype)	United States
PD 267	*Dieffenbachia* sp.	Netherlands
PD 345	*Dieffenbachia* sp.	Netherlands
PD 663	*Dieffenbachia* sp.	Netherlands
PD 481	*Kalanchoë blossfeldiana* Poelln. 'Seraya'	Netherlands
PD 489	*Kalanchoë blossfeldiana* Poelln. 'Seraya'	Netherlands
PD 551	*Kalanchoë blossfeldiana* Poelln. 'Pollux'	Netherlands
PD 554	*Kalanchoë blossfeldiana* Poelln. 'Pollux'	Netherlands
PD 593	*Kalanchoë blossfeldiana* Poelln.	Netherlands
PD 594	*Kalanchoë blossfeldiana* Poelln. 'Forty Niner'	Netherlands
PD 598	*Kalanchoë blossfeldiana* Poelln.	Netherlands
P 664	*Kalanchoë blossfeldiana* Poelln.	Netherlands
PD 665	*Kalanchoë blossfeldiana* Poelln. 'Yellow Nugget'	Netherlands
86/122	*Kalanchoë blossfeldiana* Poelln. 'Tessa'	Netherlands
NCPPB 533	*Philodendron* sp.	United States
PD 471	*Philodendron erubescens* K. Koch & Aug. 'Emerald King'	Netherlands
PD 487	*Philodendron erubescens* K. Koch & Aug. 'Emerald King'	Netherlands
PD 550	*Scindapsus pictus* Hassk.	Netherlands
PD 552	*Scindapsus pictus* Hassk.	Netherlands
PD 553	*Scindapsus pictus* Hassk.	Netherlands
PD 226	*Solanum tuberosum* L. (= IPO[d] 502)	Netherlands
PD 472	*Solanum tuberosum* L.	Netherlands
PD 482	*Solanum tuberosum* L. 'Ostara'	Netherlands
PD 483	*Solanum tuberosum* L. 'Ostara'	Netherlands
PD 484	*Solanum tuberosum* L. 'Bintje'	Netherlands
PD 490	*Solanum tuberosum* L.	Netherlands
PD 499	*Solanum tuberosum* L.	Netherlands
PD 581	*Solanum tuberosum* L. 'Element'	Netherlands
81/310	*Solanum tuberosum* L.	Netherlands
84/924	*Solanum tuberosum* L. 'Saturna'	Netherlands
CFBP 2015	*Solanum tuberosum* L.	Netherlands
NCPPB 2538	*Zea mays* L. (pathotype)	United States
NCPPB 1066	*Zea mays* L.	Egypt
NCPPB 2347	*Zea mays* L.	Italy
NCPPB 2476	*Zea mays* L.	Malaysia
Erwinia carotovora subsp. *atroseptica*		
PD 230	*Solanum tuberosum* L.	Netherlands
Erwinia carotovora subsp. *carotovora*		
PD 544	*Solanum tuberosum* L.	Netherlands

[a] PD, culture collection Plant Protection Service, Wageningen, The Netherlands.
[b] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.
[c] CFBP, Collection Française de Bactéries Phytopathogènes, Angers, France.
[d] IPO, culture collection Research Institute for Plant Protection, Wageningen, The Netherlands.

bacteria of the antisera used. For determination of O and H specificities of sera, see references 37 and 38. Bacteria were not heat treated to retain flagellar antigens. For preparation method of sera 7621 and 7676, see reference 45. Flagella types are indicated with numbers following a system similar to the one developed for *Escherichia coli* (32), instead of letters as used by Samson et al (40). (Also see Figure 1.)

Reisolations from inoculated plants were checked by slide agglutination or indirect immunofluorescence (IF) with antisera 7621 and 7676. Serogroup and flagella type were determined by IF using sera in a 400-fold dilution (15); Nordic (Tilburg, The Netherlands) Sw/AR serum labeled with fluorescein-isothiocyanate in a 100-fold dilution served as a conjugate. For IF, 0.1 ml of a 24-hr NB culture was added to 1-ml sterile distilled water. From this mixture, 25 μl was placed on a 10-well multitest slide, air dried, and very gently heat fixed. Motility of cultures was checked just before preparation of the slides. IF slides were examined with a Leitz (Wetzlar, West Germany) Ortholux microscope with epifluorescent light. Fluorescing objects were photographed using Kodak (Rochester, NY) high-speed (400 ASA) Ektachrome daylight film. Extinction of fluorescence under ultraviolet light was retarded by adding 100 mg *p*-phenylenediamine to the glycerophosphate buffer, pH 7.6.

TABLE 2. Antisera used against *Erwinia chrysanthemi* and homologous strains

Serum no.	Sero-group	Flagella type	Homologous strains	Source
84	1	1	CFBP[a] 2013 *Dahlia*	R. Samson, Angers
158	1	1	CFBP 1888 *Solanum tuberosum*	R. Samson, Angers
174	1	2	CFBP 2048 *Chrysanthemum morifolium*	R. Samson, Angers
35	2	3	CFBP 1236 *Chrysanthemum maximum*	R. Samson, Angers
71	3	...[b]	CFBP 1451 *Musa paradisiaca*	R. Samson, Angers
7621	1	1	IPO[c] 502 = PD[d] 226, *S. tuberosum*	IPO, Wageningen
7676	1	1	PD 97 *C. morifolium*	IPO, Wageningen

[a] CFBP, Collection Française de Bactéries Phytopathogènes, Angers, France.
[b] No antibodies against flagella.
[c] IPO, culture collection Research Institute for Plant Protection, Wageningen, The Netherlands.
[d] PD, culture collection Plant Protection Service, Wageningen, The Netherlands.

Pathogenicity tests. *Zea mays* L. hybrid LG 11 from seed, *Solanum tuberosum* L. 'Bintje' seed, class S, *Kalanchoë blossfeldiana* Poelln. 'Calypso' rooted cuttings, and *Philodendron erubescens* K. Koch & Aug. 'Emerald King' rooted cuttings were used as test plants. Four plants were placed in 75-cm-long trays with sterilized potting soil and grown in a computer-controlled, insect-free glasshouse.

Each bacterial strain was inoculated into four plants per test plant species with a 23 G hypodermic needle, using a 10^7 cell.ml^{-1} suspension in sterile physiological saline (PS) of a 24-hr NA culture. When the suspension had to be poured into the leaf whorl (1 ml/leaf whorl), 0.5%, v/v, of Tween 20 was added (22). Bacterial concentration was adjusted using a $BaSO_4$ standard (25). Control plants (four per test plant species) were inoculated as described above, using sterile PS.

From each test plant species, reisolations were made which were confirmed by colony morphology on YPGA and slide agglutination and/or IF.

Corn—first inoculation. Corn plants were inoculated when the third true leaf had just uncurled. Two plants were inoculated in the stem base and two others into the leaf whorl. After inoculation, plants were placed for seven days at a day and night temperature of 28 C, a day length of 14 hr, and 95–99% RH. After 7 days, night temperature was lowered to 18 C, and relative humidity was lowered to 85–90%.

Corn—second inoculation. When it appeared that stem inoculations of corn were unsuccessful, stem-inoculated plants

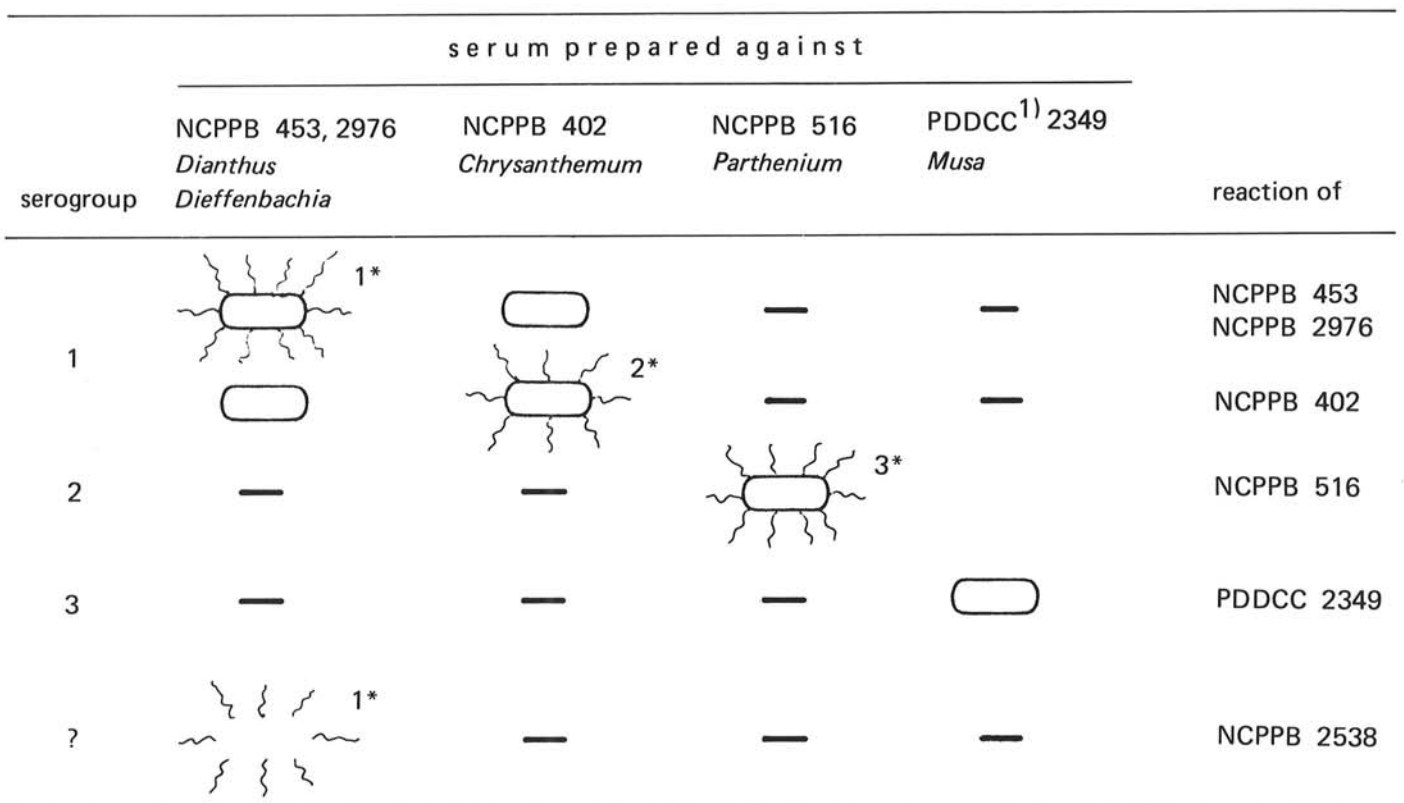

Fig. 1. Definition of serogroups and flagella types of *Erwinia chrysanthemi* according to Samson et al (40). —— Solid line = no reaction; ⬭ = reaction with O antigens; ⬭ = reaction with flagellar antigens. NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, U.K. PDDCC, Plant Diseases Division Culture Collection, Auckland, New Zealand. 1*, 2*, 3* = flagella types.

were once more inoculated into the leaf whorl 10 days after the first inoculation. All plants were then kept 3 days at a day and night temperature of 28 C, a day length of 14 hr, and 95–99% RH. Thereafter, night temperature was lowered to 18 C and relative humidity was lowered to 85–90%.

Potato. Each plant, about 30 cm long, was inoculated in a few stems at soil level and several higher places. After inoculation, plants were treated as described for corn (second inoculation).

Philodendron and Kalanchoë. All plants were inoculated in the leaf whorl at the third true leaf stage (Philodendron) or in the stem at the fifth true leaf stage (Kalanchoë) and by a needle into leaf parenchyma (intercellular infiltration of about 0.5 cm tissue) and a main vein of a leaf. After inoculation, plants were treated as described for corn (second inoculation).

RESULTS

Identification of strains as *Erwinia* species. All strains tested were Gram-negative, motile, straight rods, measuring 0.5–0.7 × 1–3 μm. Two colony types could be distinguished after 2 days growth on YPGA: gray to cream-yellow, glistening, irregular colonies with an irregular margin and raised center, up to 4 mm in diameter; and gray to cream-yellow mucoid to very mucoid lobate or round colonies with an entire margin, flat, sometimes with a raised center. There was no correlation between colony morphology and pathogenicity, virulence, or biovar.

All strains were oxidase negative and catalase positive, showed fermentative metabolism of glucose and hydrolyzed pectin and gelatin, produced alkali from sodium citrate, produced H_2S (PD 267 and 345 [culture collection Plant Protection Service, Wageningen, The Netherlands] negative), reduced nitrate, and used D(+)cellobiose, galacturonate, D-mannitol, L(+)rhamnose, salicin, and L(+)serine (PD 499 and CFBP 1200 [collection Française de Bactéries Phytopathogènes, Angers, France] negative; PD 483 and 490 doubtful) as a carbon source. No strain formed reducing substances from gluconate (*Z. mays* strains and PD 382 doubtful) or used D-asparagine or gallate (*Z. mays* strains doubtful) as a carbon source. On the basis of these results, all strains except NCPPB 533 (National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.) belonged to the genus *Erwinia*.

Identification of strains as *E. chrysanthemi*. Tests used for identification and reactions of strains are listed in Table 3. The reference strains PD 230 and PD 544 were identified as *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora*, respectively. All other strains were identified as *E. chrysanthemi* except NCPPB 533. This strain, deposited as *E. chrysanthemi* in the NCPPB collection, is a nonpectolytic member of the Enterobacteriaceae, and further determination (Janse and Ruissen, *unpublished*) showed it to be *Klebsiella pneumoniae* (*K. aerogenes*). Because it was nonpathogenic in all tests, it is excluded from further discussion.

According to Perombelon and Hyman (34), strains of *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, and *E. chrysanthemi* can be separated by pectinolysis on CVP medium at different temperatures. Only *E. chrysanthemi* should give pectinolysis at 37 C. All strains of *E. chrysanthemi* indeed showed pectinolysis at this temperature (Table 4). However, *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* also showed moderate pectinolysis.

Classification of *E. chrysanthemi* strains into biovars. Results of biovar-discriminating tests according to Samson et al (40), presented in Table 4, show good correlation with her results. Tests described by Thomson et al (43) proved to be variable in our hands (Table 5). Growth on D-asparagine, D(+)cellobiose, ethanol, and L(+)tartrate deviated too much from their findings; reactions with gallate and β-lactose were not clear; and growth reactions on salicin and L-serine were not discriminative enough. Perhaps tests for utilization of γ-aminobutyrate and L-proline can be added to the tests of Samson et al (40), because they gave better results. There was a pronounced difference in pectinolysis at 37 C between biovar 3 strains (strong) and those of biovars 1, 5, 6, and 7

TABLE 3. Tests used for identification of *Erwinia carotovora* subsp. *atroseptica* (*E.c.a.*), *E. carotovora* subsp. *carotovora* (*E.c.c.*), and *E. chrysanthemi* (*E. chr.*) and reactions of 43 strains (including reference strains)[a]

Test	*E.c.a.* (1 strain)	*E.c.c.* (1 strain)	*E. chr.* (41 strains)
Cellulolytic activity	−	−	+
Growth in 5% NaCl	+w	+	−
Indole production	−	−	+
Reducing substances sucrose	+	−	−[b]
Alkali production Na-malonate	−	−	+
Growth on: D(+)maltose	+	−	−[c]
palatinose	+	−	−
D(+)trehalose	+	+	−[d]

[a] +, positive; −, negative; w, weak.
[b] PD 382 and 487 (culture collection Plant Protection Service, Wageningen, The Netherlands), and NCPPB 1066, 2347, 2476, and 2976 (National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.) weakly positive.
[c] NCPPB 402 and 2347 weakly positive.
[d] NCPPB 2347 weakly positive.

TABLE 4. Tests used for identification of *Erwinia chrysanthemi* biovars, reactions according to Samson (40) (first column under each biovar), and reactions of 40 strains tested (second column under each biovar)[a]

	Biovar													
Test	1 (1 isolate)		2 (4 isolates)		3 (11 isolates)		4[b]	5 (7 isolates)		6 (1 isolate)		7 (17 isolates)	
Growth at 39 C	−	−	+	+[c]	+	+	+	+	v	+	+	−	−
Arginine degradation	+	+	−	−	−	−	−	+	+	−	−	+	v
Cis-aconitate	+	−	−	−	+	+[d]	−	+	v	+	+	−	−[e]
D(−)arabinose	−	−	+	+	+	+	+	−	−	−	−	−	−
5-Ketogluconate	−	−	−	−	−	−[f]	+	−	−	−	−	−	−
Inulin	+	+	−	−	−	−	−	+	+	−	−	+	+
Mannitol	+	+	+	+	+	+	−	+	+	+	+	+	+
Melibiose	+	+	−	−	+	+	+	+	+	+	+	−	−
Raffinose	+	+	−	−	+	+	+	+	+	+	+	−	−
D(−)tartrate	+	d	−	−	−	−[g]	+	−	−	−	−	+	v

[a] +, Positive; −, negative; v, variable; d, doubtful.
[b] Reactions are from Samson's data (40) only; biovar 4 was not tested in this study.
[c] PD 345 and 267 (culture collection Plant Protection Service, Wageningen, The Netherlands) negative, NCPPB 2976 (National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.) doubtful.
[d] PD 471, 487, 550 negative; PD 430 and 552 doubtful.
[e] PD 499 positive.
[f] NCPPB 2538 positive.
[g] PD 471 and 552 weakly positive.

TABLE 5. Additional tests used for identification of *Erwinia chrysanthemi* biovars, reactions according to Thomson et al (43) (first column under each biovar), and reactions of 40 strains tested (second column under each biovar)[a]

Test	Biovar 1 (1 isolate)		2 (4 isolates)		3 (11 isolates)		5 (7 isolates)		6 (1 isolate)		7[b] (17 isolates)
γ-Aminobutyrate	−	−	−	−	+	+[c]	−	−	−	+	−
D-asparagine	−	−	−	−	+	−	+	−	+	−	−
D(+)cellobiose	−	+	+	+	+	+	+	+	+	−	+
Ethanol	−	+	−	v	+	v	+	+	+	+	+
Gallate	−	−	−	−	±	±/−	−	−	v	−	−
Glycerate	+	+	−	+	+	+	−	+	+	+	+
β-Lactose	−	−	−	−	+	d[d]	+	−	+	d	−
L-proline	−	−	−	−[e]	+	−[f]	−	−[g]	−	w	−
Salicin	v	+	+	+	+	+	+	+	+	+	+
L-serine	v	+	v	+	+	+	+	+	+	−	+[h]
L(+)tartrate	−	+	+	+	v	+[i]	−	+	+	+	+[j]
Pectinolysis at 37 C		w		++[k]		+++/++		w[l]		+	d/w/+

[a] +, positive; −, negative; d, doubtful; v, variable; w, weak.
[b] Data are from this study only.
[c] PD 471, 487, 550, 552 (culture collection Plant Protection Service, Wageningen, The Netherlands) negative.
[d] NCPPB 2347 and 2538 (National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.) and PD 382 positive.
[e] PD 663 weak.
[f] PD 430 and 487 negative.
[g] NCPPB 402 weak.
[h] PD 483 and 490 doubtful.
[i] PD 552 negative; PD 550 doubtful.
[j] PD 594 negative.
[k] NCPPB 2976 +++.
[l] NCPPB 402 ++.

TABLE 6. Classification of 31 *Erwinia chrysanthemi* strains from The Netherlands and NCPPB 2339[a] into biovars

Biovar	Strains[b]	Deviations in test results from those of Samson et al (40)
1	None	
2	*Dieffenbachia* (3/3)	PD 267 and 345[c]: no growth at 37 C.
3	*Aechmea* (1/1); *Aglaonema* (1/1); *Philodendron* (2/2); *Scindapsus* (3/3)	PD 430 and 552: cis-aconitate doubtful. PD 471, 487, 550: cis-aconitate negative.
4	None	
5	*Solanum tuberosum* (4/11) *Cichorium intybus* (1/1)	PD 482, 484, 490, 499, 82/21 no growth at 39 C; pathotype NCPPB 402: cis-aconitate negative.
6	NCPPB 2339 from *Chrysanthemum*	
7	*Solanum tuberosum* (7/11); *Kalanchoë* (10/10)	PD 472 L(+)arginine and D(−)tartrate negative; other *Solanum* isolates and most *Kalanchoë* strains D(−)tartrate doubtful; *Kalanchoë* strains L(+)arginine negative.

[a] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.
[b] Per number of strains tested.
[c] PD, culture collection Plant Protection Service, Wageningen, The Netherlands.

TABLE 7. Serogroup and flagella type of 40 *Erwinia chrysanthemi* strains[a]

Serogroup-flagella type	Biovar	Strains and host
O1:H1	1	CFBP[b] 1200 (*Dianthus*)
	2	NCPPB[c] 2976 (*Dieffenbachia*)
	3	PD[d] 382 (*Aechmea*)
	5	PD 482, 484, 490, 499 (potato), 82/21 *Cichorium intybus*
	7	PD 226, 472, 483, 581, 81/310, 84/924, CFBP 2015 (potato); PD 481, 489, 551, 554, 593, 594, 598, 664, 665, 86/122 (*Kalanchoë*)
O1:H2	5	NCPPB 402 (*Chrysanthemum*)
O1:H?	2	PD 267, 345, 663 (*Dieffenbachia*)
	3	PD 471, 487 (*Philodendron*); PD 550, 552, 553 (*Scindapsus*); PD 430 *Aeglaonema*; NCPPB 2347 (corn)
O2:H3	...[e]	...
O3	...	...
O?:H1	3	NCPPB 2476, 2538 (corn)
	6	NCPPB 2339 (*Chrysanthemum*)
O?:H?	3	NCPPB 1966 (corn)

[a] NCPPB 533 excluded.
[b] CFBP, Collection Francaise de Bactéries Phytopathogènes, Angers, France.
[c] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.
[d] PD, culture collection Plant Protection Service, Wageningen, The Netherlands.
[e] Strains with this reaction were not found.

(moderate to weak). Biovar 2 strains were intermediate. There was a correlation between pectinolysis at 37 C and growth at 39 C (Tables 4 and 5). Final classification is given in Table 6. Biovars 1 and 4 were not found among strains from The Netherlands.

Classification of *E. chrysanthemi* strains into serogroups and flagella types. Results of serological classification on the basis of O and H antigens are given in Table 7. Serogroups 2 and 3 and flagella type 3 were not found among Dutch *E. chrysanthemi* strains. The combination O?:H1 (? = unknown serogroup) was not only found for corn strains NCPPB 2538 and 2476 (biovar 3), but also for NCPPB 2339 (biovar 6). The most frequent combination was O1:H1 found in biovars 3, 5, and 7 (Fig. 2).

Dutch strains from *Dieffenbachia* (biovar 2) deviated in flagella type from the pathotype NCPPB 2476 from the United States.

Corn strains (biovar 3) from different geographical origin were serologically heterogeneous. The other biovar 3 strains also reacted differently: PD 382 from *Aechmea* reacted as O1:H1, cross-reacting as O2:H3; and *Philodendron*, *Scindapsus*, and *Aglaonema* strains reacted as O1:H?.

The pathotype of biovar 5 (NCPPB 402 from chrysanthemum) reacted as O1:H2; other biovar 5 strains from cichory and potato reacted as O1:H1.

Strains of biovar 7 from potato and *Kalanchoë* reacted very homogenously as Ol:Hl. Strain PD 593 weakly cross-reacted with serum 174 (Ol:H2).

E. carotovora subsp. *atroseptica* strain PD 230 strongly cross-reacted with O1 antibodies of serum 7621 and weakly with those of serum 84 and 174 and also with O2 antibodies of serum 35.

Pathogenicity tests. Table 8 lists the reactions of 40 strains of *E. chrysanthemi* in four test plant species (Fig. 3).

Corn. Stem inoculation proved to be unsuccessful (except for NCPPB 1066). Leaf-whorl-inoculated plants developed symptoms after 3 days. Symptoms were curling, yellowing, and wilting of leaves, red discoloration of the main vein, sometimes wet, glassy to brown necrotic leaf spots, wet, glassy, yellow to reddish brown stem lesions, later with a white, sunken necrotic center (Fig. 4), malformation of newly formed leaves, and finally a total yellow-brown necrosis and death of the plant. Yellowish to red bacterial slime was sometimes observed.

Potato. Two days after stem inoculation of potato, the first symptoms appeared, consisting of fading of leaf color, wilting, and epinasty of leaf petioles. Disease development was rapid under conditions of high temperature and humidity. Further symptoms were yellowing of tip leaflets, growth retardation, dark brown to black stem lesions and pith discoloration, hollow stems and yellow to reddish brown vascular discoloration. Eventually stems collapsed completely and died (Fig. 5).

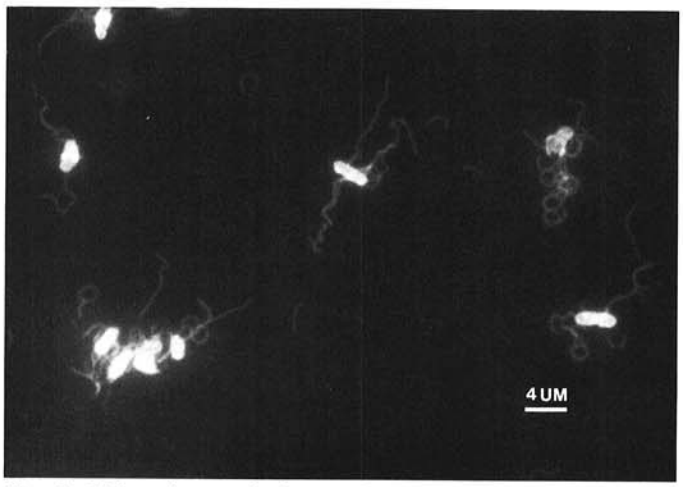

Fig. 2. Light microscopic immunofluorescent reaction of *Erwinia chrysanthemi* 82/21 (*Cichorium*, biovar 5, O1:H1) with serum 158 (anti O1:H1). Homologous reaction, cells and flagella visible.

TABLE 8. Pathogenicity of 40 *Erwinia chrysanthemi* strains for four test-plant species[a]

Original host	Biovar	*Zea mays*	*Solanum tuberosum*	*Philodendron*	*Kalanchoë*
Aglaonema	3	++	+	++	–
Aechmea	3	+++	++	++	–
Philodendron	3	++/+++	+++	++	–
Dieffenbachia	2	++	+++[b]	+/++	–[c]
Scindapsus	3	+++	+++	++/+++	–
Chrysanthemum	5/6	+++	++	++	–
Z. mays	3	+++	++	++	–
Dianthus	1	++	+	–	–
Kalanchoë	7	+++	+/++/+++	–	++/+++
S. tuberosum	5/7	++/+++	++/+++	–[d]	+/++/+++
Cichorium intybus	5	++	+++	–	++

[a] –, negative; +, moderate; ++, high; +++, very high.
[b] PD 663 (culture collection Plant Protection Service, Wageningen, The Netherlands) ++.
[c] NCPPB 2476 (National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.) +.
[d] PD 581 +.

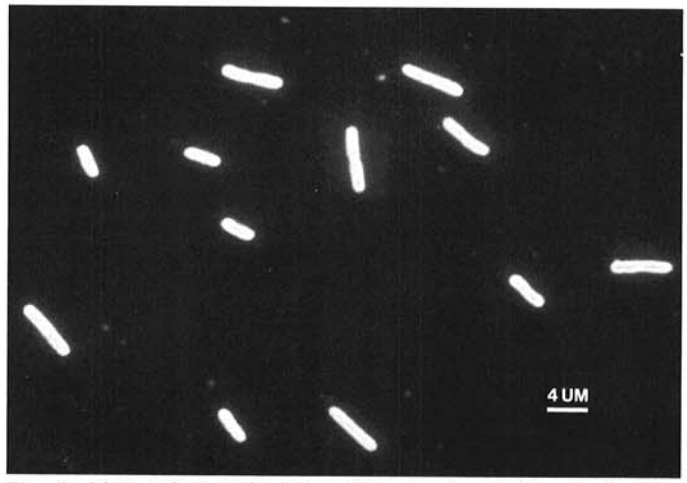

Fig. 3. Light microscopic immunofluorescent reaction of *Erwinia chrysanthemi* PD 482 (culture collection Plant Protection Service, Wageningen, The Netherlands) (*Solanum tuberosum*, biovar 5, O1:Hl) with serum 174 (anti Ol:H2). Reaction with homologous Ol antigens only, flagella not visible.

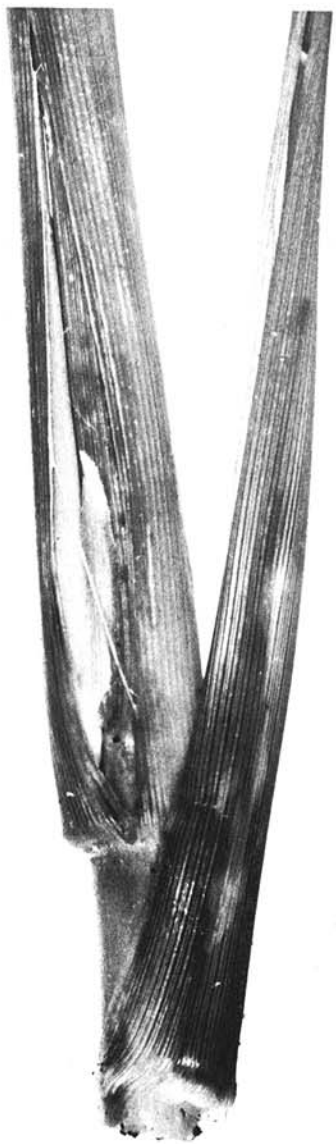

Fig. 4. *Zea mays* 'LG 11,' 13 days after inoculation with *Erwinia chrysanthemi* PD 499 (culture collection Plant Protection Service, Wageningen, The Netherlands) (*Solanum tuberosum*, biovar 5). Wet, yellow to reddish brown stem lesions and leaf lesions with cracked tissue.

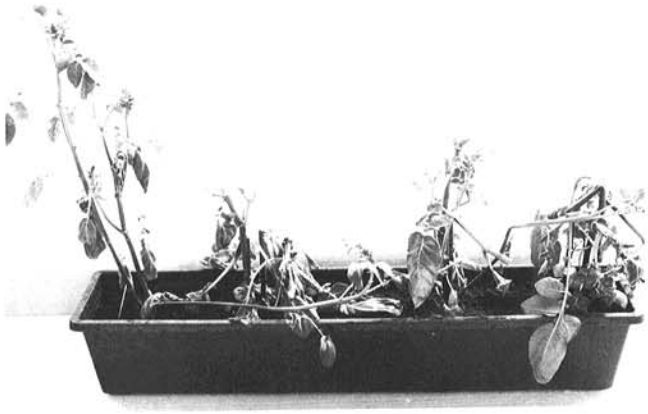

Fig. 5. *Solanum tuberosum* 'Bintje,' 3 days after inoculation with *Erwinia chrysanthemi* PD 550 (culture collection Plant Protection Service, Wageningen, The Netherlands) (*Scindapsus*, biovar 3). Severe wilting and collapse of plants.

Fig. 6. *Kalanchoë blossfeldiana* 'Calypso,' 5 wk after inoculation with *Erwinia chrysanthemi* PD 593 (culture collection Plant Protection Service, Wageningen, The Netherlands) (*Kalanchoë*, biovar 7). Left: wilted leaf with characteristic wrinkle pattern. Right: healthy turgescent leaf.

Philodendron. Only leaf infiltration was successful in *Philodendron*. Two days after inoculation, rapidly developing symptoms became visible. Infection stopped at the petiole, except for PD 552 and 553 (*Scindapsus*), 487 (*Philodendron*), and 430 (*Aglaonema*). In the last case, the base of the plant became infected and growth reduction and malformation took place. Symptoms were rapidly developing glassy, later brown, to black irregular leaf spots, sometimes surrounded by a yellow halo. At the leaf underside, sometimes drops of yellowish bacterial slime could be observed on the leaf spots. Strains NCPPB 2538 (corn), PD 226 (potato), and PD 481 (*Kalanchoë*) only showed a yellow spot at the infiltrated zone. The bacterium could be isolated from these spots only in PD 481, 14 days after inoculation.

Kalanchoë. Only stem inoculations were successful. In contrast to the other test plants, on *Kalanchoë* disease development was very slow. Only 2 wk after inoculation, the first wilting symptoms were found. Symptoms were wilting (consisting of flabby leaf tips and a peculiar wrinkle pattern at the leaf underside [Fig. 6]), red discoloration of the wilting leaves, growth reduction, yellow to reddish brown vascular discoloration, blackening of the pith (Fig. 7), and finally a stem rot resulting in death of the plant. Roots also were infected, becoming brittle and brown necrotic.

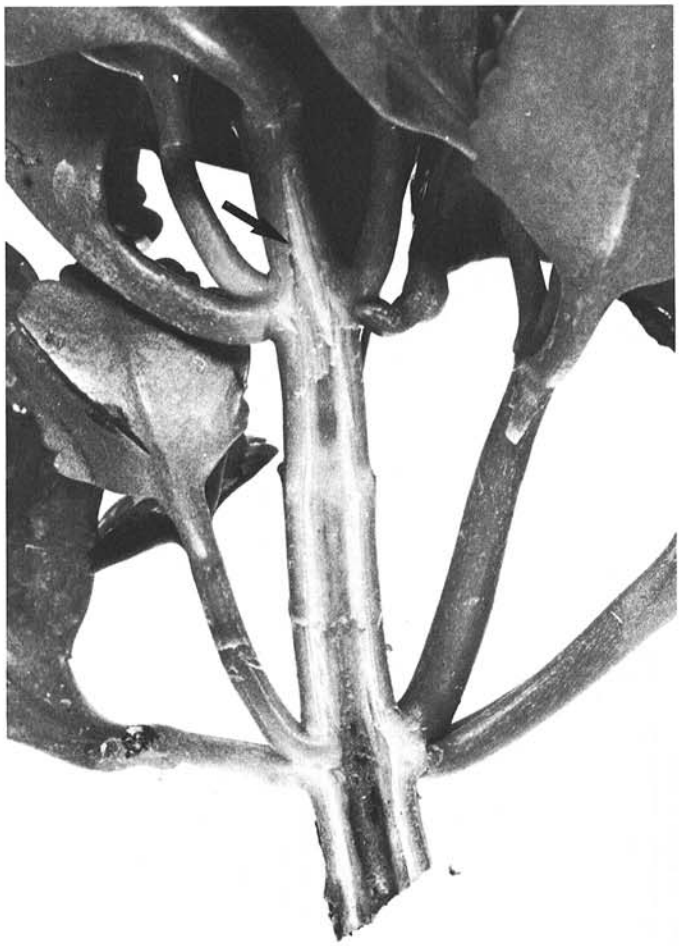

Fig. 7. *Kalanchoë blossfeldiana* 'Calypso,' 5 wk after inoculation with *Erwinia chrysanthemi* 82/21 (*Cichorium*, biovar 5). Reddish brown discoloration of the pith and vascular discoloration in upper parts of the plant (arrow).

DISCUSSION

All strains used in this study were phenotypically identified as *Erwinia* species, except the *Philodendron* strain NCPPB 533, which appeared to be *Klebsiella pneumoniae* (*K. aerogenes*). Forty strains used were identified as *E. chrysanthemi*. *E. chrysanthemi* strains PD 269 and 345 from *Dieffenbachia* did not show H_2S production, also observed by Dye (16) for some strains from pineapple, corn, *Dianthus*, and *Dieffenbachia*.

We studied carbon source utilization by growth on agar instead of pH shift in a liquid medium (40). Results of both methods were well correlated, except for organic acids which were retested in the medium of Collins and Lyne modified by Webb (46). Four of the seven biovars described (40) (biovars 2, 3, 5, and 7) were found to be present among *E. chrysanthemi* strains from The Netherlands. No new biovars were found if one accepts a minimal difference of two test results between biovars. Thus, *Kalanchoë* strains, which are arginine-negative instead of positive, could be classified as biovar 7 (based on a potato isolate). However, one potato strain (PD 472) also was arginine-negative. Strains from the same host from different geographical regions can belong to the same biovar. Danish (14) and Dutch (this study) *Kalanchoë* strains both belong to biovar 7. Corn strains from different parts of the world belong to biovar 3 (this study, 38). This may be due to spread of *E. chrysanthemi* with a common source of plant propagation material. Strains from the same host plant from different geographical regions also can belong to different biovars. Potato strains from The Netherlands were classified in biovars 5 and 7, those from France in biovars 1 and 7 (39), those from Taiwan in biovar 6 (19), and Australian and Peruvian strains in biovar 3

(6,11). One chrysanthemum strain (NCPPB 402, U.S.) belongs to biovar 5, another (NCPPB 2339, U.K.) to biovar 6. Contamination from neighboring crops may play a role in this diversity of biovars on the same host. This was indicated for biovar 3 potato strains in Australia (6), where corn was suspected as a source. Unfortunately these potato strains were not compared serologically with Australian corn strains.

The cross-reaction of *E. carotovora* subsp. *atroseptica* strain PD 230 indicates the presence of common antigens between *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*. Cross-reactions between *E. chrysanthemi* and *E. chrysanthemi* subsp. *atroseptica* and also *E. carotovora* subsp. *carotovora* have been described earlier (27,37). Perhaps the presence of glucose as well as fucose in lipopolysaccharide of both *E. chrysanthemi* and *E. carotovora* subsp. *atroseptica* may be the basis of certain cross-reactions (5,9). Cross-reactions of flagellar antigens of *E. carotovora* subsp. *atroseptica* or *E. carotovora* subsp. *carotovora* with *E. chrysanthemi* sera (8) were not found; however, only one strain of *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* was tested. Corn strains from different geographical regions are serologically very heterogeneous (this study, 13,39). Sixteen strains from the same region were homogeneous (36). As in earlier studies (13,47), no clear correlation was found between serogroup-flagella type and biovar or original host. The serological diversity of *E. chrysanthemi* may cause problems when serological detection methods are used for this pathogen.

All potato and *Kalanchoë* strains reacted very uniformly as O1:H1. However, a complete serological identity can be determined only by absorption of anti O1:H1 sera with these strains. With cross-absorbed sera (so-called factor sera), more subtle subdivisions within O groups can be made (37). A possibility of subdivision is shown by PD 267 (O1:H?) which did not react with two of the five O1 sera used. Also, within flagella types, subdivisions could be possible as demonstrated by the different reactions of the same H1 strain with different H1 sera at a 400-fold serum dilution (from negative to strongly positive) and by the cross-reaction of PD 598 (O1:H1) with H2 serum. Absence of (good) IF reaction with all sera by NCPPB 1066, 2476, 2538 (corn), and 2339 (chrysanthemum) indicates the presence of more than three serogroups, the absence of H reactions of some corn strains and strains from greenhouse ornamentals, the presence of new flagella types. Dickey et al (13) and Yakrus and Schaad (47) distinguished four serovars, based on antisera against heat-treated whole cells and a membrane-protein complex, respectively. For their classification they used strains different from those used by Samson (37,38), making comparison difficult. Our results and those of Samson (37,38) show the possibility of developing a very refined serological determination of *E. chrysanthemi* strains. Furthermore, it is of utmost importance for diagnostic and epidemiological research to have a central laboratory producing (factor) sera, testing them with many strains, and distributing them pooled or separately.

In pathogenicity tests, corn had to be leaf-whorl inoculated with Tween 20 added to the suspension to obtain uniform results. This also was found earlier (18,39). Schaad and Brenner (41) using stem inoculation were unable to prove pathogenicity of *E. chrysanthemi* strains from six different host plants (including corn strains) for corn. Pathogenicity for corn of strains from five of those host plants was demonstrated in our tests using leaf-whorl inoculation. Dickey (11) also mentioned conflicting results among different pathogenicity tests. For *Philodendron* only, leaf infiltrations were successful.

Using *Kalanchoë* as a test plant, only stem and petiole inoculations were positive, which also was found for *Dieffenbachia* (31). This once more demonstrates the importance of a carefully selected pathogenicity test for *E. chrysanthemi* in host-range studies.

All Dutch *E. chrysanthemi* strains were pathogenic for the potato cultivar Bintje and the frequently planted corn hybrid LG 11. Until now *E. chrysanthemi* has not been found on corn in The Netherlands, but our findings indicate that a diseased ornamental or potato crop may be a potential threat for corn.

Inoculation results of *Kalanchoë* and *Philodendron* show a previously unknown division between strains. All strains from corn and indoor plants were pathogenic on *Philodendron*, but strains from the temperate plants *C. intybus*, potato, and *Kalanchoë* were not. On *Kalanchoë* the reverse was true. The division of "warm" and "temperate" strains also was found using growth at 39 C and pectinolysis on a pectate medium at high temperature (37 C) as differentials.

It is very plausible that strains from *C. intybus*, potato, and *Kalanchoë* have adapted to crops grown in temperate climates and that their adaptation can be physiologically, biochemically, and pathologically recognized. However, more research should be conducted to sustain this hypothesis.

The overall conclusion about classification of *E. chrysanthemi* strains is that still much work has to be done using other strains from different regions. The naming of separate *E. chrysanthemi* types as pathovars (48) is not of much advantage because these types are not exclusive at all for a certain host plant (also see 40). Perhaps the following construction is worth consideration: *E. chrysanthemi* (*Dieffenbachia*), biovar 2, O1:H1. This construction is also usual in medical bacteriology for Enterobacteriaceae such as *Salmonella* and *Escherichia* (24,32).

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