Occurrence of Alfalfa Mosaic, Hydrangea Ringspot, and Tobacco Ringspot Viruses in *Hydrangea* spp. in British Columbia

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ABSTRACT

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A survey for manually transmissible viruses in commercial stocks of *Hydrangea* spp. was conducted in British Columbia in 1983. Of 73 stocks of various cultivars of *H. macrophylla* sampled, alfalfa mosaic virus (AMV), hydrangea ringspot virus (HRSV), or both viruses were detected in 5, 18, and 10 stocks, respectively. HRSV occurred in two of two sampled stocks of *H. serrata* 'Bluebird,' and tobacco ringspot virus (TobRSV) was found in two of 23 sampled stocks of *H. paniculata* 'Grandiflora.' Evidence suggested that each of these viruses occurred universally in some stocks. No viruses were detected in four other species of hydrangea sampled. This is the first report of virus infection in *H. serrata* and *H. paniculata* and the first definitive report of AMV and TobRSV infecting *Hydrangea* spp. Adopting simple control measures might markedly improve the quality of some stocks, especially those partially infected with both AMV and HRSV.

In August 1982, we observed conspicuous foliar symptoms in florists' hydrangea (*Hydrangea macrophylla* Ser.) at two garden centers in Victoria, BC. Employees at these establishments reported customer complaints about the appearance and growth of such plants. Subsequent tests (*unpublished*) showed that each plant sampled was infected with either hydrangea ringspot virus (HRSV) or both HRSV and alfalfa mosaic virus (AMV). HRSV has been detected commonly in florists' hydrangea in other countries (6,13,16,22) and is believed to be distributed worldwide (14). A virus thought to be AMV was previously isolated from florists' hydrangea in Italy (3), but this diagnosis was inconclusive, because it was based solely on symptomatology and cross-protection tests. Several additional viruses have been reported to infect florists' hydrangea (1,2,5,19,20,22) and other species of hydrangea (15,17,18).

There has been no previous report on the identity or incidence of viruses infecting *Hydrangea* spp. in Canada. This, combined with our preliminary finding of HRSV and AMV in florists' hydrangea, prompted a survey for viruses infecting commercially grown *Hydrangea* spp. in British Columbia. Results of this survey are reported in this paper.

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MATERIALS AND METHODS

Survey. Samples of all species and cultivars of hydrangea maintained at each of 31 nurseries and garden centers on Vancouver Island and the lower mainland of British Columbia were collected in June and July 1983. Nearly all hydrangea growers listed in the *B.C. Nursery Trades Association Trade Directory 1979–80* were included in the survey. At the time of sampling, age and growth stage of hydrangeas varied widely from 1-yr-old vegetative plants to relatively old treelike plants in full bloom. Each sample consisted of one young leaf per sampled plant. When circumstances permitted, samples were collected systematically from each type of hydrangea present (usually two samples from plants with symptoms and/or two or three samples from symptomless plants). In some instances, however, sampling was unavoidably selective because of the arrangement of stock or limited numbers of plants. Each sample was kept singly in a sealed polyethylene bag in a cool environment until tested for infectivity (1–4 days later).

Infectivity assay. Each sample was ground in a chilled mortar with 3 ml of 0.5% bentonite + 0.5% K_2HPO_4 (23), and the extract was rubbed on four corundum-

dusted basal leaves of a young plant of *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Reyn., and tobacco (*Nicotiana tabacum* L. 'White Burley'). The first two species are susceptible to HRSV; the third is not (11). With the exception of an apparently rare ilarvirus detected recently in florists' hydrangea in England (20), other viruses reported from *Hydrangea* spp. are capable of infecting tobacco. Test plants were maintained in a heavily shaded greenhouse at mean daily minimum and maximum temperatures of 16 and 30 C, respectively. Final observations of test plants for symptoms were made about 4 wk after inoculation.

Serology and electron microscopy. Antisera to AMV were obtained from E. M. J. Jaspars, D. Z. Maat, and G. I. Mink; antisera to other viruses tested for were obtained from D. Z. Maat.

Immunosorbent electron microscopy (ISEM), similar to that described elsewhere (21), was used to identify HRSV. Extracts for this procedure were prepared by grinding one to three local lesions excised from selected inoculated leaves of *G. globosa* (or occasionally from *C. amaranticolor*) in a mortar with 10 volumes (w/v) of 0.06 M potassium phosphate buffer, pH 7.0 (6-PB). Antiserum coating of grids (backed with collodion-carbon films) and subsequent virus particle trapping and decoration on appropriate droplets of HRSV antiserum (diluted 1/100 in 6-PB) or extract were for 15, 15, and 10 min, respectively. Grids were washed after each treatment in a stream (about 2 ml) of 6-PB, given a final wash in a stream of distilled water, then stained with 2% uranyl acetate.

Immunodiffusion tests were used to identify other viruses isolated in this study. The diffusion medium was prepared with 1% Noble agar, 0.85% NaCl, and 0.02% NaN_3 in 0.01 M potassium phosphate buffer, pH 7.0 (1-PB). Extracts for these tests were obtained by grinding inoculated or systemically infected leaves from selected test plants in a mortar with 1-PB (1 ml/g of tissue) and filtering the juice through cheesecloth; unless mentioned otherwise, extracts were from tobacco. Antisera were tested undiluted and diluted one-half and one-fourth with 0.85% NaCl. Appropriate controls with normal serum and extracts from uninoculated plants were included in all serological tests.

Leaf-dip preparations were either shadowed with platinum-palladium alloy (80:20) or fixed with 4% glutaraldehyde and negatively stained with 2% sodium phosphotungstate, pH 6.8. ISEM and leaf-dip preparations were examined with a Philips 300 or Hitachi H-600 electron microscope.

Nomenclature and terminology. In referring to species of hydrangea, we have followed the nomenclature used by Hillier (10). Cultivar names of hydrangea were obtained from tags on sampled stocks. Some of the names of cultivars of florists' hydrangea mentioned in this paper have not been registered with the international registration authority; some may be mentioned in nursery lists or catalogs, but this alone does not constitute valid registration (E. McClintock, *personal communication*).

The frequency of virus infection in sampled stocks is designated as either "universal" or "partial"; these respective terms indicate that all or a portion of the samples tested were found to be infected.

RESULTS AND DISCUSSION

In 1983, viruses were detected by infectivity assays in three of seven species of commercially grown *Hydrangea* in 16 of 31 (52%) establishments located throughout the region surveyed. Of 123 stocks of *Hydrangea* spp. sampled, 37 (30%) were found to be virus-infected.

In florists' hydrangea, viruses were detected in 33 of 73 (45%) sampled stocks. HRSV, AMV, or both viruses were found in 18, 5, and 10 stocks, respectively. Stocks of Glowing Ember, Revelation, and Trophy were probably universally infected with either or both of these viruses, whereas other named cultivars were apparently infected to a lesser degree (Table 1). Previous workers (6,13,16,22) have reported that HRSV occurs universally in some cultivars of florists' hydrangea but in varying proportions of plants of other cultivars.

Detection and identification of HRSV was generally based on development of necrotic local lesions in inoculated leaves of *G. globosa* and subsequent detection of decorated flexuous rods in extracts of these lesions by ISEM (Fig. 1). In some instances, however, inoculum from florists' hydrangea induced only a few chlorotic local lesions in *C. amaranticolor* and failed to infect *G. globosa*. In these instances, HRSV was identified in lesion extracts of the former species. In *G. globosa*, lesions induced by AMV and putative spontaneous lesions (8), which appeared occasionally, were usually smaller or more variable in size than those induced by HRSV. However, if the cause of lesions in *G. globosa* was unclear, selected lesions were examined for virus particles in leaf-dip preparations and/or by ISEM.

During this study, *N. benthamiana* Domin was found to be a systemic host for HRSV. Although symptoms in infected plants were generally mild and of little diagnostic value, this species may be useful as a propagation host for purifying the virus. Only *Primula maculoides* Franch. has been reported to be a good systemic host for propagating HRSV, but infected plants do not develop symptoms (11).

In a metal-shadowed leaf-dip preparation from infected *N. benthamiana*, the normal length of particles (211 measured) of one isolate of HRSV was 515 nm, a value similar to that reported for this virus by Bercks and Brandes (4).

Most isolates of AMV were detected serologically in extracts from inoculated or systemically infected leaves of tobacco with AMV antiserum from one donor (G. I. Mink); strongest reactions were generally obtained from leaves showing the most severe symptoms. A few isolates of AMV, however, could not be detected

Table 1. Incidence of hydrangea ringspot virus (HRSV) and alfalfa mosaic virus (AMV) infection in commercial stocks of florists' hydrangea in British Columbia in 1983

Cultivar	No. of stocks tested	No. of stocks infected universally (U) or partially (P)[a]							
		HRSV		AMV		HRSV and AMV			
		U	P	U	P	UU[b]	UP	PU	PP
All Summer Beauty	4	...	...	...	...	...	...	...	...
Bark Pink	2	...	1	...	...	...	...	...	...
Blue Prince	1	...	...	...	...	...	...	...	...
Bluewave	1	...	1	...	...	...	...	...	...
Domotoi	1	...	...	...	...	...	...	...	...
Glowing Ember	3	3	...	...	...	...	...	...	...
Kuhnert	2	...	1	...	...	...	...	...	...
Merritt's Beauty	2	...	...	...	1	...	1	...	...
Merritt's Blue	1	...	...	...	...	...	...	...	1
Merritt's Pride	2	...	...	...	...	...	...	...	...
Merritt's Supreme	1	...	...	...	1	...	...	...	...
Mariesii	1	...	...	...	...	...	...	...	...
Merveille	3	2	1	...	...	...	...	...	...
Nikko Blue	2	...	1	...	...	...	...	...	...
Nikko Pink	1	...	1	...	...	...	...	...	...
Pink Beauty	2	1	1	...	...	...	...	...	...
Raymond	1	...	...	...	...	...	...	...	...
Revelation	3	1	...	1	...	...	...	1	...
Sister Teresa	3	...	...	...	...	1	...	...	...
Strafford	1	...	...	...	...	...	...	...	...
Todi	3	...	...	...	1	...	...	...	...
Trophy	3	1	...	1	...	1	...	...	...
Variegata	3	...	...	...	...	...	...	...	...
Unidentified	27	3	...	...	...	...	3	...	2
Total	73	11	7	2	3	2	4	1	3

[a] Viruses detected by infectivity assays in all (U) or a portion (P) of samples from plants (generally two to four) of each stock and subsequently identified serologically.

[b] For mixed infections, individual letters of dual symbols refer to the incidence of HRSV and AMV infection, respectively.

in tobacco leaf extracts with AMV antisera from all three donors, but these were readily detected in leaf extracts from other species such as *C. quinoa* Willd. or *N. benthamiana*.

Sizes were determined of 598 particles comprising several isolates of AMV (in *G. globosa* and tobacco) obtained from florists' hydrangea. In negatively stained leaf-dip preparations, 95% of these particles were 21–66 nm long and the remainder were longer (up to 107 nm); the mean particle width was 22 nm. The particle length distribution (not shown) was multipeaked, as expected for AMV, but only three peaks, probably representing bottom, middle, and top b components (12), were clearly resolved.

Red, purple, or brown ringspots in lower leaves were the most common symptoms in florists' hydrangea infected with HRSV. These symptoms also occurred in plants infected with both HRSV and AMV but were accompanied by conspicuous mottling or mosaic on apical foliage. Plants infected with AMV alone showed only the latter symptoms (Fig. 2). Leaf rugosity and distortion were also observed frequently (generally on only a few leaves per plant), but these symptoms apparently were not associated solely with virus-infected plants.

Inoculation tests have shown that HRSV is responsible for ringspot symptoms in florists' hydrangea (11). Similar tests must still be conducted to determine if AMV causes mosaic in this species or if infection by both AMV and HRSV induces more severe effects than infection by either virus alone.

HRSV was also detected in two of two sampled stocks of *H. serrata* Ser. 'Bluebird,' and evidence indicated that both stocks were universally infected. Symptoms in infected plants were similar to those in florists' hydrangea infected with the same virus. Although this is the first report of virus infection in *H. serrata*, infection of this species by HRSV is not surprising, because it is believed to be a hybrid with *H. macrophylla* as one of the parents (10).

A virus with spherical particles (30 measured in a negatively stained leaf-dip preparation) averaging 29 nm in diameter was isolated from two of 23 sampled stocks of *H. paniculata* Sieb. 'Grandiflora.' Evidence indicated that this virus infected one stock partially and the other stock universally. The only apparent symptoms of infection were observed in the latter stock; foliage on all plants was faintly mottled and leaves on some plants showed very faint chlorotic ringspots. The virus infected all three species of test plants and was subsequently identified serologically in extracts from each species as tobacco ringspot virus (TobRSV). The virus did not react with antisera to arabis mosaic, tomato black ring, or tomato ringspot (TomRSV) viruses, nepoviruses previously found to infect *Hydrangea* spp. (5,17,18). This is the first report of a virus infecting *H. paniculata* and the first definitive report of TobRSV infecting *Hydrangea*. A virus presumed to be TobRSV was isolated previously from florists' hydrangea, but its identity was based solely on symptomatology and cross-protection tests (2). The fallibility of these criteria for identifying viruses is discussed by others (9).

Symptoms induced in three species of test plants by each of the viruses detected in this survey are noted in Table 2. Because a number of factors can influence the detection of viruses by infectivity assays, the incidence of infection in *Hydrangea* spp. determined by these assays may be conservative.

There was no evidence of virus infection in the following species and cultivars of *Hydrangea*: *H. arborescens* L., *H. paniculata* 'Floribunda,' *H. petiolaris* Sieb. & Zucc., *H. quercifolia* Bartr., and *H. sargentiana* Rehd. (10, 2, 9, 3, and 1 stocks sampled, respectively).

Most stocks of florists' hydrangea that we found to be virus-infected, including those infected with AMV, were imported from the United States. Although AMV has not been reported from florists' hydrangea in the United States, its occurrence there in this species may simply have gone undetected. Mosaic in florists' hydrangea was reported in the United States as early as 1952 (7). Although TomRSV was presumably

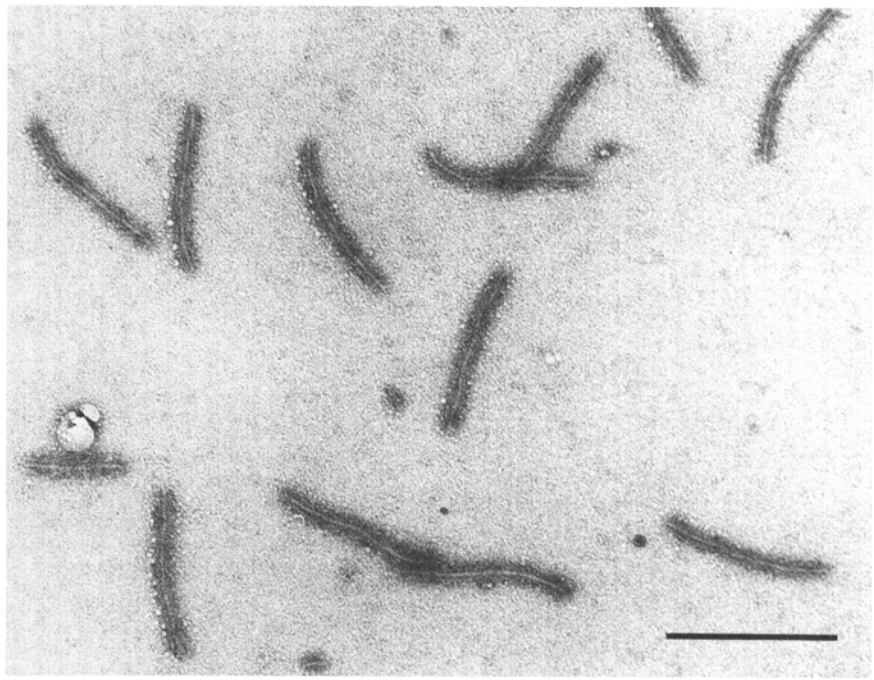

Fig. 1. Immunosorbent electron micrograph showing trapped hydrangea ringspot virus (HRSV) particles decorated with antibodies to HRSV. Scale bar = 500 nm.

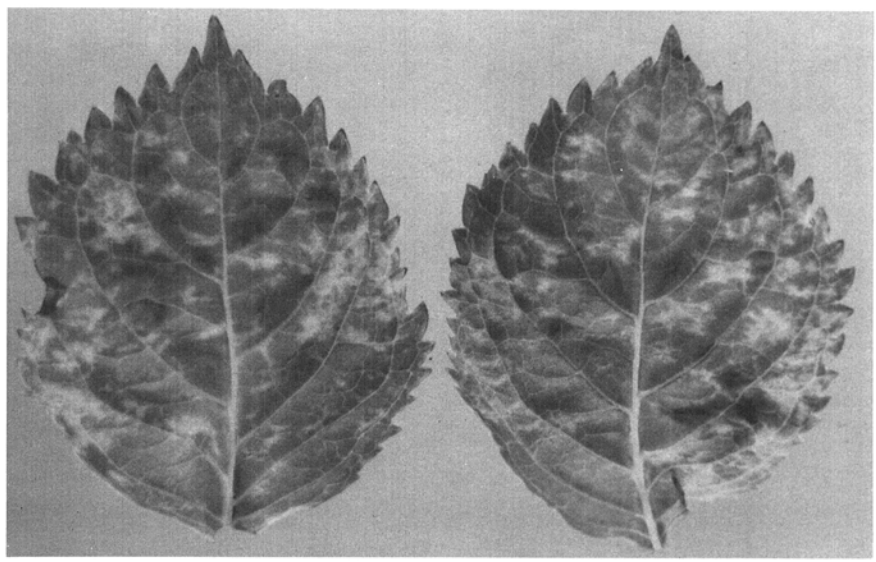

Fig. 2. Vein yellowing, mottle, and mosaic symptoms in leaves from florists' hydrangea infected with alfalfa mosaic virus.

Table 2. Range of symptoms in three species of test plants manually inoculated with each of three viruses from *Hydrangea* spp.

Virus[a]	Leaves[b]	Symptoms in test plants[c]		
		Gomphrena globosa	*Chenopodium amaranticolor*	*Nicotiana tabacum* 'White Burley'
AMV	I	CL,NL,CR,M	CL,NL,M,SI	CL,NL,NR
	AI	CL,NL,CR,M,SI	CL,CR,M,SI	CL,NL,NR,M,SI
HRSV	I	CL,NL	CL,NL,CR	0
	AI	0	0	0
TobRSV	I	CL,CR	CL,NL	CR,NR
	AI	CR,M,SI	CL,M,SI	CR,NL,NR

[a]AMV = alfalfa mosaic virus, HRSV = hydrangea ringspot virus, and TobRSV = tobacco ringspot virus.
[b]I = inoculated, AI = above inoculated.
[c]CL/NL = chlorotic/necrotic lesions, CR/NR = chlorotic/necrotic rings or ringspots, M = mottle and/or mosaic, SI = symptomless infection, and 0 = not infected.

isolated from some plants with this symptom (5), this virus was apparently not responsible for all mosaiclike patterns in this species (7).

Brierley and Lorentz (6) suggested that because most cultivars of florists' hydrangea in the United States were tolerant to HRSV, propagation practices were not designed to protect newer, less tolerant cultivars from becoming infected. In England, however, Hollings (11) considered symptoms in some cultivars infected with this virus to be sufficiently severe to warrant both roguing affected plants and using clean cutting knives during propagation. The relatively severe symptoms we observed in florists' hydrangea infected with both AMV and HRSV suggest that propagators might markedly improve the quality of some partially infected cultivars by employing similar control measures. Plants free of HRSV have been produced from infected florists' hydrangea by heat therapy or meristem-tip culture (16), but similar techniques have yet to be evaluated for eliminating AMV from this species.

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LITERATURE CITED

1. Allen, T. C., McMorran, J. P., and Locatelli, E. A. 1983. Isolation of tomato spotted wilt virus from hydrangea and four weed species. Plant Dis. 67:429-431.
2. Anderson, C. W. 1958. Tobacco ringspot virus on florists' hydrangea. Plant Dis. Rep. 42:932-933.
3. Belli, G. 1968. Ricerche su una clorosi nervale dell'Ortensia. Phytopathol. Mediterr. 7:70-71.
4. Bercks, R., and Brandes, J. 1961. Vergleichende serologische und elektronenmikroskopische Untersuchung des Weisskleemosaik-Virus, des Hydrangea ringspot virus und des Kartoffel-X-Virus. Phytopathol. Z. 42:45-56.
5. Brierley, P. 1954. Symptoms in the florists' hydrangea caused by tomato ringspot virus and an unidentified sap-transmissible virus. Phytopathology 44:696-699.
6. Brierley, P., and Lorentz, P. 1957. Hydrangea ringspot virus, the probable cause of "running-out" of the florists' hydrangea. Phytopathology 47:39-43.
7. Brierley, P., and Smith, F. F. 1952. A ringspot virus from hydrangea infectious to herbaceous plants. Plant Dis. Rep. 36:382-383.
8. Francki, R. I. B. 1967. Effect of high light intensities on spontaneous and virus-induced local lesions in *Gomphrena globosa*. Phytopathology 57:329.
9. Hamilton, R. I., Edwardson, J. R., Francki, R. I. B., Hsu, H. T., Hull, R., Koenig, R., and Milne, R. G. 1981. Guidelines for the identification and characterization of plant viruses. J. Gen. Virol. 54:223-241.
10. Hillier, H. G. 1974. Hilliers' Manual of Trees and Shrubs. 3rd ed. David & Charles, Newton Abbot, England. 575 pp.
11. Hollings, M. 1958. Hydrangea ringspot in Britain. J. Hortic. Sci. 33:275-281.
12. Hull, R. 1969. Alfalfa mosaic virus. Adv. Virus Res. 15:365-433.
13. Koenig, R. 1971. Virusuntersuchungen an Hortensien 1970. Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschweig) 23:41-42.
14. Koenig, R. 1973. Hydrangea ringspot virus. Descriptions of Plant Viruses. No. 114. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
15. Kristensen, H. R. 1964. Plantesygdome i Danmark. Virussygdomme. Tidsskr. Planteavl 67:591-597.
16. Paludan, N., and Christensen, M. 1973. Hortensie-ringmosaik-virus i hortensie (Hydrangea macrophylla Ser.). Diagnostik, kortlaegning, termoterapi og meristemkultur. Tidsskr. Planteavl 77:1-12.
17. Schmelzer, K. 1970. Untersuchungen an Viren der Zier-und Wildgeholze. 7. Mitt.: Weitere Befunde an *Buddleja, Viburnum, Caryopteris* und *Philadelphus* sowie Viren an *Leycesteria, Chionanthus, Ribes, Hydrangea, Syringa, Spiraea* und *Catalpa*. Phytopathol. Z. 67:285-326.
18. Schmelzer, K. 1974. Untersuchungen an Viren der Zier-und Wildgeholze. 8. Mitt.: Neue Befunde an *Forsythia, Hydrangea*, und *Philadelphus* sowie Viren und Virosen an *Rhamnus, Centaurea, Galvezia, Cistus, Forestiera, Abeliophyllum, Celastrus, Staphylea* und *Crambe*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2, 129:139-168.
19. Tamura, M., and Komuro, Y. 1967. The mosaic disease of hydrangea caused by cucumber mosaic virus. Ann. Phytopathol. Soc. Jpn. 33:27-31.
20. Thomas, B. J., Barton, R. J., and Tuszynski, A. 1983. Hydrangea mosaic virus, a new ilarvirus from *Hydrangea macrophylla* (Saxifragaceae). Ann. Appl. Biol. 103:261-270.
21. Van Regenmortel, M. H. V. 1982. Serology and Immunochemistry of Plant Viruses. Academic Press, New York. 267 pp.
22. Welvaert, W., and Samyn, G. 1973. De verspreiding van het hydrangea ringspot virus bij de *Hydrangea macrophylla* (Thunb.) DC. Meded. Fac. Landbouwwet. Rijksuniv. Gent 38:1647-1654.
23. Yarwood, C. E. 1972. Virus transmission from *Chenopodium amaranticolor*. Plant Dis. Rep. 56:1085-1086.