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Hydrangea virescence. I. — Description of the disease and its transmission to the differential host plant *Catharanthus roseus* by *Cuscuta subinclusa*

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SUMMARY

A virescence disease has recently been observed in France on *Hydrangea macrophylla*. This ornamental plant is cultivated in a rather particular way, comprising two successive phases : "production of mature plants" and "production of flowering plants". The possible period of disease inception and symptom appearance has been studied with respect of these two phases. Symptoms have been compared with those already described from different localities in Europe and America. Transmission of the pathogen to the differential host periwinkle (*Catharanthus roseus*) through dodder (*Cuscuta subinclusa*) revealed that the virescence observed in France was different from that in Belgium and had some similarities with the grassy shoot disease of *Gladiolus*.

Additional key words : MLO, culture-types.

RÉSUMÉ

La virescence de l'hortensia. I. — Description de la maladie et transmission par Cuscuta subinclusa à un hôte différentiel, Catharanthus roseus.

Les symptômes de la virescence de l'hortensia récemment apparue en France sont comparés à ceux décrits dans divers pays d'Europe et aux U.S.A. Leur période d'apparition et les risques de contamination sont décrits en tenant compte de la culture particulière de l'hortensia, caractérisée par deux stades très différents : la culture « en vert » et le « forçage ». La transmission à *Catharanthus roseus* par *Cuscuta subinclusa* montre que la souche observée en France diffère de celle décrite en Belgique et présente de grandes analogies avec les germes fins ou Aster Yellow du glaïeul.

Mots clés additionnels : *Hydrangea*, MLO, techniques culturales.

I. INTRODUCTION

Hydrangea macrophylla Thunb. (Saxifragaceae), a native of Japan, was first introduced into Europe at the end of the eighteenth century. In France it has now become one of the major horticultural crops with an annual production reaching 5 million plants.

While the fungal diseases of *Hydrangea* have been well described and effectively controlled, virescence disease still remains problematic for horticulturists. For the last few years, the incidence of virescence has been observed to increase consistently, as indicated by the following data : per 100 000 plants, virescence was noted yearly on 1-3 plants before 1983, 15-20 in 1983, and as many as 400 in 1984. These data, however,

remain underestimated as it was difficult to verify the occurrence of the disease in all the nurseries which purchase mature plants.

Several cases of virescence on *Hydrangea* have already been reported from different countries in Europe : Germany (MUTH, 1933 ; MÜLLER, 1971), Italy (BELLI *et al.*, 1973), Belgium (WELVAERT *et al.*, 1975). It has also been reported in the United States (BRIERLEY, 1944 ; HEARON *et al.*, 1976 ; LAWSON & SMITH, 1980a,b).

In view of the economic importance of this plant in France and the threat to its successful production by the disease, a research programme was launched at Versailles with the aim of clarifying the present disease picture. Thus, attempts were made to describe the

disease symptoms on *Hydrangea* and their sequential appearance during the growing period. Furthermore, based on the differential host reaction obtained by passing the pathogen to periwinkle (*Catharanthus roseus* (L.) G. Don) through dodder (*Cuscuta subinclusa* Dur. & Hilg.), an attempt was made to classify the virescence type under investigation among the different diseases of the yellows type.

II. MATERIALS AND METHODS

A. Materials

Healthy and virescent *H. macrophylla* cultivar « Merveille » plants, already flowering in a « forcing » nursery in the suburb of Paris, were used in the present study.

For a comparative study of the diseases appearing in Belgium and France, Prof. WELVAERT's laboratory supplied virescent *Hydrangea* plants from the Gent region and periwinkle plants showing the yellows symptoms achieved by inoculation through *C. subinclusa* from virescent *Hydrangea*.

At Versailles, clones of periwinkle propagated by stem cuttings were used to compare the types of virescence with our existing reference collection.

B. Methods

1. Grafting

Periwinkle plants from Belgium were maintained in the glasshouse for reference and the disease was transmitted to our clone of periwinkle by grafting.

2. Transmission of the disease by dodder

C. subinclusa seedlings were propagated and allowed to parasitize young healthy *Nicotiana tabacum* L. var. *xanthi* plants, for 12 days in the glasshouse. These tobacco plants, along with dodder, were then brought and kept in contact with virescent *Hydrangea* cymes for 10 days, so that the parasite could establish connections by *Hydrangea*. The tobacco plants were subsequently disconnected from the *Hydrangea* and replaced with healthy periwinkle plants. After another 10 days of close contact of the dodder on *Hydrangea* and periwinkle, the periwinkles were taken away from the *Hydrangea*. A few days later, the dodder shoots were also removed from the host plants.

III. OBSERVATIONS AND RESULTS

A. Symptoms on *Hydrangea*

The virescence studied in France was characterized by the abnormalities observed on cymes (Plate I,1). In affected plants, either all the cymes, some of them, or in certain cases, only a single cyme were seen to have abnormal flowers. In a single cyme, all the grades of colour breaking, virescence, phyllody and proliferation could be observed. Abnormalities were evident in all flower parts and comprised in particular reduction in size and progressive greening of petaloid

sepals, irrespective of their initial original colour ; blue, pink or white. Later, these sepals became phylloid. Colour breaking was sometimes restricted to some sepals of the flower (Plate I,1, right side). Proliferations were common in the pistil and, in some cases, the latter was replaced by vegetative shoots (Plate I,1, left side and 2). In most cases the foliage did not show any abnormality in colour, shape or size. However, it occasionally happened that some lower branches remained vegetative and produced leaves showing epinasty and flavescence (Plate I,3).

« Rosette » disease, which is often mistaken for the virescence could be distinguished from the latter by its symptoms : reduction in leaf size, shortening of internodes and inhibition of apical growth frequently followed by necrosis. Rosette affected either the whole plant or a sector of it. Normal foliage was sometimes observed to emerge from diseased portions.

B. Period of appearance of symptoms (Schedule)

The growing period, which is very particular to *Hydrangea*, comprises two successive phases (fig. 1) :

1. The « production of mature plants »

This phase starts from the propagation of cuttings and lasts until the maturation of the plants, just when they are entering their reproductive phase.

Figure 1
Two phases of *Hydrangea* cultivation : I. Production of mature plants. II. Production of flowering plants.
Les deux phases de la culture de l'hortensia. I. Culture en vert. II. Forçage.

In France, it is exclusively restricted to the Loire Valley from Orléans to Angers.

Three types of « production of mature plants » can be distinguished depending on the duration of the growing period, number of pinches, number of cymes produced by each plant and the average shoot height (table 1).

Defoliation terminates the « production of mature plants » phase, and makes the plant go into dormancy.

TABLE 1
Type of production of mature plants.
Type de production en vert.

« Production of mature plants » : different types		Duration (months)	Number of pinches	Number of cymes produced per plant	Average height of the shoot (cm)
Traditional culture	long	16-18	2	3-7	50
	short	9-12	1	1-3	50
Accelerated culture of miniature plants		5-7	2	7-15	25

2. « Production of flowering plants » or forcing

This allows the plants to flower and is independent of the three types of « production of mature plants ». Both phases may be seen through by the same nursery, but normally they are handled by different ones. Forcing is done in different parts of France. Dormancy is broken by cold treatment of at least 2 weeks. Plants are then transferred to glasshouses maintained at about 20 °C. The symptoms of the virescence usually appear only in the forcing phase, after one month of keeping the plants in the glasshouse. In order to have continuous flower production from January till June, forcing is practised at regular intervals and the symptoms appear accordingly. Forcing, which lasts for about two months at the beginning of winter, progressively decreases to one month at the end of spring. The appearance of symptoms only in the forcing phase may give rise to conflicts between the nurseries producing either « mature » or « flowering » plants.

C. Transmission by dodder (Plate I,4 and 5)

Transmission of the *Hydrangea* virescence to the periwinkle plant through the dodder was successful. The symptoms obtained on periwinkle were characteristic of the aster-yellows group (Plate I,4). First visible symptoms were observed about 40 days after connecting the diseased *Hydrangea* and healthy periwinkle. Within the following two months, success of disease transmission was noted to be 75 %. The dodder showed no symptoms. Transmission tests to periwinkle were also performed in order to compare our local type of virescence with that from Belgium (Plate I,5). The symptoms were observed carefully throughout their development and the results have been presented in table 2.

TABLE 2

Comparison of symptoms on Catharanthus roseus obtained through dodder transmission of Hydrangea virescence types of France and Belgium.

Comparaison des symptômes obtenus sur Catharanthus roseus après transmission par la cuscute des types français et belge de virescence de l'hortensia.

	French type	Belgian type
Beginning of symptom appearance :	Sepals little modified or unmodified ; petals virescent and reduced ;	Calyx hypertrophied ; corolla tube shortened or absent ; petals virescent and reduced in size.
Retention of floral morphology	corolla lobes pointed and upright ; corolla tube swollen and ruptured at places due to protruding style.	
Progression of symptoms :	Petals reduced and phylloid ; phyllody of stamens and pistil.	Calyx hypertrophied ; corolla tube absent ; petal margin curled towards its adaxial surface ; petals star shaped ; no proliferation through pistil.
Aberration in floral morphology		
Later stages of symptom development :	Pistil replaced by vegetative shoot ; general yellowing in complete plant ; witches' broom aspect ; upright growth of excessively sprouted lateral shoots ; newly emerging shoots thin, wiry and pale yellow with reduced foliar surface, interveinal chlorosis (Plate I,4).	Either no flowers or if produced, flowers green and small with no proliferations ; plants more dense ; internodes shortened ; dormant apical buds ; excessive sprouting of axillary buds ; in the upper part of branches the leaves more in number but reduced in size, interveinal chlorosis (Plate I,5).
Disappearance of floral morphology		

IV. DISCUSSION

The results mentioned above confirmed the existence of a virescence disease on *Hydrangea* in France. The pathogen could readily be passed through dodder from diseased *Hydrangea* to healthy periwinkle plants. It produced symptoms characteristic of aster yellows on periwinkle, used as a differential host plant.

The *Hydrangea* virescence type studied in France was different from the types described in other European countries. Symptoms of *Hydrangea* virescence described in Belgium (WELVAERT *et al.*, 1975) were characterized by dwarfing accompanied by vein yellowing or reddening of the foliage of diseased plants. The type we studied showed no symptoms on the foliage except that, in certain cases only, epinasty and flavescence were noted in some branches. In addition to these differences in symptoms, some differences were noted in the reaction of dodder as well as in the differential host plant periwinkle. For instance, with the French type, no symptoms were observed on dodder while the Belgian type involved witches' broom symptoms on dodder during the transmission process (WELVAERT *et al.*, 1975). On periwinkle, the French type produced aster yellows symptoms unlike the Belgian one which induced strobil-like symptoms. These results suggest a difference in the host reservoirs in the two countries. These

reservoirs seem to be different from those of clover phyllody as the symptoms obtained on periwinkle by the transmission of *Hydrangea* virescence through dodder in the two countries, and by leafhoppers in Belgium (SAMYN *et al.*, 1982), were different from clover phyllody.

American workers have, however, distinguished 3 types of the disease : severe, mild and intermediate, differing in the magnitude of the effect on foliage and flowers.

These types were maintained by successive graftings (LAWSON & SMITH, 1980b). The French type of virescence, studied here, seems to have some analogy with the mild American type because of the frequent absence of foliar symptoms.

The results concerning the development of aster yellows symptoms in periwinkle by transmitting the French type of *Hydrangea* virescence support the serological work of CLARK & DAVIES (1984) and indicate that the *Hydrangea* virescence agent is distinct from clover phyllody and has some relationship with the aster yellows agent. However, the work of CLARK & DAVIES (1984) did not concern itself with the existence of different types of *Hydrangea* virescence while the present work confirms the existence of two different types of virescence in France and Belgium.

The French type of *Hydrangea* virescence produced the aster yellows symptoms in periwinkle already described in the grassy shoot disease of *Gladiolus* sp. by ALBOUY (1966). It would thus be interesting to examine whether the insects : *Euscelis incisus* (E. plebejus) Kirschbaum and *Macrosteles quadripunctulatus* Kirschbaum, vectors of grassy shoot of *Gladiolus*, could play the same role in the transmission of *Hydrangea* virescence. It would then be possible to investigate whether the open air period during the « production of mature plants » could be the time when the plants are infected via the insect vectors.

The present results show that symptoms of virescence usually appear only in the forcing phase, one

month after keeping the plants in the glasshouse. Infection could happen long before the forcing period, i.e. during the « production of mature plants ». Therefore, any prophylactic or control measure against the disease, should take into consideration the importance of this period in the disease development process.

Transmission by dodder has presented many difficulties in the case of *Hydrangea* virescence. The failure of American workers (HEARON *et al.*, 1976) to transmit the disease to periwinkle could be explained by the fact that they used *Cuscuta campestris* Yunck as a means of transmission. Similar negative result were obtained in Belgium with either *C. campestris* or *Cuscuta europea* L. (Prof. W. WELVAERT, pers. comm.). Transmission by *C. subinclusa*, which did give positive results, has however encountered some major difficulties as *Hydrangea* is not a favourable host for the growth of dodder. When *C. subinclusa* was allowed to grow on *Hydrangea* for a longer period, the parasite degenerated and eventually died. On the other hand, when the exposure time was very short, the parasite was unable to produce its haustoria and thus failed to transmit the disease. It is necessary to use very young branches of dodder and to make regular observations on their parasitism on *Hydrangea*. These difficulties seem to limit the use of dodder in wide-scale transmission tests, e.g. in routine diagnosis necessary for a phytosanitary selection programme.

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Plate 1.

Comparison of symptoms obtained on *Catharanthus roseus* by transmission through dodder of the French and Belgian types of *Hydrangea* virescence disease.

Comparaison des symptômes obtenus sur *C. roseus* lors de la transmission par cuscute des virescences française et belge de l'hortensia.

1 — Cyme of a virescent *Hydrangea macrophylla* plant : flowers showing normal pink colour, colour breaking, complete virescence, phyllody of petaloid sepals and replacement of pistils by vegetative shoots ; progression of symptom development from right to left.

Cyme d'hortensia atteint de virescence ; on observe simultanément la coloration rose normale, le verdissement plus ou moins intense, la complète virescence et la phyllodie des sépales pétaloïdes. Sur certaines fleurs, une poussée végétative se développe à l'emplacement du pistil. Noter la progression des symptômes de droite à gauche sur le cliché.

2 — A single flower of *Hydrangea* cyme showing greening in petaloid sepals and petals ; replacement of pistil by vegetative shoot. Fleur isolée d'hortensia ; virescence des sépales pétaloïdes et des pétales et remplacement du pistil par une pousse végétative.

3 — *Hydrangea* plant showing normal (left) and virescent (right) cymes. Progressive reddening of petaloid sepals in virescent cyme, some of the branches of the plant remaining stunted with leaves showing flavescence and epinasty.

Plant d'hortensia montrant une cyme normale (à gauche) et une virescente (à droite). Sur cette dernière, on observe le rougissement des sépales pétaloïdes. Quelques rameaux, dont la croissance est réduite, portent des feuilles jaunissantes et incurvées vers le bas (épinastie).

4 — *Catharanthus roseus* showing aster yellows symptoms obtained by the transmission of the French *Hydrangea* virescence disease through dodder *Cuscuta subinclusa*. Little modified or unmodified sepals, reduced and phyllod petals, upright and pointed petal-lobes, phyllod stamens and pistil, at places vegetative shoot replacing the pistil ; upright growth of excessively sprouted lateral shoots ; thin, wiry and pale yellow newly emerging shoots with reduced foliar surface.

Catharanthus roseus présentant des symptômes caractéristiques de l'aster yellow, à la suite de la transmission de la souche française de virescence de l'hortensia par *Cuscuta subinclusa* : taille réduite des sépales comparable à celle des témoins, pétales réduits et phylloïdes dont les lobes sont dressés et aigus, étamines et pistil phylloïdes, pousses végétatives développées à l'emplacement des pistils, prolifération intense des rameaux latéraux à croissance érigée, nouvelles pousses formées grêles, filamenteuses, d'un jaune pâle avec une surface foliaire réduite.

5 — *Catharanthus roseus* showing stolbur-like symptoms obtained by the transmission of the Belgian *Hydrangea* virescence disease through *Cuscuta subinclusa*. Hypertrophied calyx, flowers with shortened or no corolla tube, star shaped petals, no proliferation through pistil ; shortened internodes, and excessive sprouting of axillary buds.

Catharanthus roseus présentant des symptômes de type Stolbur à la suite de la transmission de la souche belge de l'hortensia par *Cuscuta subinclusa* : calice hypertrophié, pétales en forme d'étoiles, le tube de la corolle étant particulièrement réduit, absence de prolifération du pistil, entre-nœuds réduits, intense prolifération des bourgeons axillaires.

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