Ethrel induced leaf senescence and increased TMV susceptibility in tobacco

Die pflanzengerontologische Wirkung des Ethrels und Zunahme der TMV-Anfälligkeit in Tabakpflanzen

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The application of Ethrel (2-chloroethylphosphonic acid) to plants can be used to regulate a number of physiological processes and may have practical or commercial value. It has been used as an ethylene producing agent in plant physiology. Its use a plant regulator was first described by WARNER and LEOPOLD (1969). The mechanism for the formation of ethylene from Ethrel has been studied. Most of the Ethrel applied to plants is eventually converted into ethylene. The release of ethylene appears to be a simple base-catalyzed reaction and does not involve any enzymatic activity in the plant (LOUGHEED and FRANKLIN 1970). Treatment of tobacco with Ethrel caused mature leaves to lose their green color and turn yellow. Tobacco leaves treated during the curing process can probably be subjected to shorter yellowing periods than the untreated leaves (STEFFENS et al. 1970). Therefore, Ethrel can be used to hasten senescence. It has been reported that an increased rate of senescence of tobacco leaves also increased susceptibility to tobacco mosaic virus (TMV) (KIRÁLY et al. 1968). Similar results have been reported with inoculated leaves after their excision (NAKAGAKI and MATSUI 1971) and after treatment with actinomycin D (LOEBENSTEIN et al. 1969, MUKHERJEE et al. 1967), chloramphenicol (KIRÁLY et al. 1968; SELA et al. 1969), ethylene (BALÁZS et al. 1969; NAKAGAKI et al. 1970; GÁBORJÁNYI et al. 1971), Ethrel (CHEO 1971) and abscisic acid (BALÁZS et al. 1973). This study was made to determine the effects on tobacco leaf susceptibility to TMV after the Ethrel treatment of the leaves.

1 Materials and methods

The U 1 strain of TMV was cultured in *Nicotiana tabacum* cv. Samsun plants. Young tobacco leaves showing typical disease symptoms of TMV were ground (1 g leaf per 10 ml buffer) with a mortar and pestle in 0,05 M phosphate buffer (pH 6,9) and the homogenate was used to inoculate the test plants. No abrasive was added to the inoculum. To determine Ethrel-induced virus susceptibility, we used *Nicotiana tabacum* L. cv. Xanthi-nc (resistant-host), and *Nicotiana tabacum* L. cv. Xanthi (susceptible-host). All tobacco plants were grown under normal greenhouse conditions, and were used for virus inoculation at the 6 to 8 leaf stage.

Ethrel (2-chloroethylphosphonic acid) at 480, 600 or 1200 µg/ml 0,1 M phosphate buffer (pH 6,5) was applied either by infiltrating one half of attached leaves (KLEMENT 1963), or by placing leaf disks (18 mm in diameter) on a layer of granulated polystyrene floated on Ethrel solution.

The latter procedure permitted good aeration of the leaf disks (BALÁZS and KIRÁLY 1972). The Ethrel solution was buffered with 1 N NaOH to pH 6,5 before dilution with 0,1 M phosphate buffer (pH 6,5).

To minimize differences in the physiological condition of the test leaves, disks were taken from each leaf in a randomized manner as described earlier (BALÁZS et al. 1973).

Virus multiplication in leaves of Xanthi tobacco was determined by local lesion assay. Six leaf disks (about 600 mg) were used for inoculation of *Nicotiana tabacum* Xanthi-nc, and the local lesions were counted. The effect of Ethrel was also estimated by measuring the diameter of local lesions (GÁBORJANYI and El-HAMMADY 1969).

2 Results and discussion

2.1 Effect of Ethrel on leaf susceptibility to TMV

To demonstrate the effect of Ethrel on leaf susceptibility to TMV, leaf disks (18 mm in diameter) were floated on Ethrel solution (pH 6,5) immediately after virus inoculation. The number of TMV lesions appearing two days after inoculation represented the increased susceptibility of leaves to TMV infection (Table 1). By raising the concentration of Ethrel, the leaf susceptibility to TMV was enhanced. The increased susceptibility of Ethrel treated leaves appears to be related to the increased senescence of the leaves. Therefore we infiltrated the attached half leaves of Xanthi-nc tobacco at different times before the inoculation. The relative lesion numbers increased as a function of time between Ethrel infiltration and TMV inoculation (Table 2).

Table 1. Effect of Ethrel on the susceptibility of Xanthi-nc tobacco leaf disks to TMV

Ethrel treatment (µg/ml)	Total number of lesion / 50,8 cm² leaf	Relative number of lesions (per cent)
0	590	100,0
600	692	117,2
1200	832	141,1

Values represent the mean of five replications. Leaf disks were floated on water or Ethrel for 2 days after inoculation. The number of lesions counted on 20 replicate disks (18 mm in diameter) represented a total leaf area of 50,8 cm². Disks were inoculated by using a glass rod. No abrasive was added to the inoculum.

Table 2. Effect of Ethrel infiltration of attached half-leaves of Xanthi-nc tobacco, susceptibility to TMV

Time between Ethrel infiltration and inoculation (h)	Total number of lesions / 51 cm² leaf		Increase of lesion number (%)
	Control (water)	Ethrel (480 µg/ml)	
0	618	887	43
24	725	1121	54
48	628	1135	80

Values represent the mean of five replications. One half of the leaf was injected with Ethrel and the opposite half with water as control. The whole leaf was then inoculated at various intervals with TMV by using a glass rod. No abrasive was added to the inoculum. The number of lesions counted on 30 disks (15 mm in diameter) represented a total leaf area of 51 cm².

2.2 Effect of Ethrel on virus multiplication

The results mentioned above in connection with enhanced virus susceptibility suggested that a certain degree of maturation or slight senescence of tissue increased virus multiplication. To examine the effect of Ethrel on virus susceptibility in an incompatible (resistant) host, TMV-infected leaf disks were floated on buffer and Ethrel solutions. Three days after inoculation the lesion diameters were measured, and used for characterising the virus multiplication (cf: GÁBORJÁNYI and EL-HAMMADY 1969). The results summarized in Table 3 showed a slight increase in lesion size. In susceptible Xanthi tobacco, the virus multiplication was measured by back inoculation 2 and 3 days after inoculation on the local lesion host (Xanthi-nc). The results after treatment showed that the virus multiplication was markedly higher in Ethrel treated leaves than in the water treated control (Table 4).

Table 3. Effect of treatment with Ethrel on TMV lesion size in Xanthi-nc tobacco leaf disk

Ethrel (µg/ml)	Lesion size (mm)	Relative lesion size
0	0,56	100
600	0,61	109
1200	0,78	139

Values represent the mean of five replications. Leaves were inoculated with TMV by using a glass rod. No abrasive was added to the inoculum. Disks from the inoculated leaves were floated on water or on Ethrel solution for two days immediately after inoculations. The mean diameter of lesions on 10 replicate disks (18 mm in diameter) was calculated after measurement under a stereoscopic microscope equipped with an ocular micrometer.

Table 4. Effect of Ethrel on the multiplication of TMV in Xanthi tobacco. Values calculated from relative virus concentration of TMV-inoculated half leaves tested on hypersensitive tobacco (Xanthi-nc)

Treatment	Lesion number	
	2 days after inoculation	3 days after inoculation
Control (buffer)	260	516
Ethrel (480 µg/ml)	490	915

Xanthi half leaves were inoculated with TMV and immediately injected with 0,1 M phosphate buffer (pH 6,5) or Ethrel solution (pH 6,5) for 48 h and 72 h. Six leaf disks (18 mm in diameter) were extracted in 3 ml 0,1 M phosphate buffer. Then half-leaves of Xanthi-nc were inoculated with the diluted extracts by using a glass rod. No abrasive was added to the inoculum. Values represent means of five replications.

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Summary

One of the most important effects of Ethrel is to hasten senescence of plants. As regards local lesions induced by viruses, our previous experience has shown that local lesion development is promoted if tissue senescence is enhanced in the host by different means. The local necrotic spots induced by TMV could be the consequence of an enhanced local senescence in host leaf tissues caused or followed by ethylene production. The aging effect of Ethrel increased both the number of lesions produced in tobacco leaves when infected with tobacco mosaic virus and also increased multiplication of the virus in the leaves. In other words, the phytogerontological effect of Ethrel increased the virus multiplication in both compatible and incompatible host-parasite relations.

Zusammenfassung

Eine der wichtigsten Wirkungen des Ethrels ist die Beschleunigung des Alterungsprozesses (Seneszens) der Pflanzen. Unsere früheren Versuche mit bestimmten Virosen zeigten, daß die Entwicklung der Lokalläsionen gefördert wird, wenn man das Altern des Gewebes auf verschiedene Art beschleunigt. Die vom TMV verursachten Lokalläsionen können Folge des gesteigerten lokalen Alterns der Blattgewebe sein, deren Ursache oder Folge die erhöhte Äthylen-Produktion sein dürfte. Die Pflanzengerontologische Wirkung des Ethrels steigerte sowohl die Anzahl der Lokalläsionen als auch die Virusvermehrung in den Blättern, wie bei den mit TMV-infizierten Tabakpflanzen ersichtlich ist. Die älter machende Wirkung des Ethrels erhöhte somit die Virusvermehrung in inkompatiblen wie in kompatiblen Wirt-Parasit-Verhältnissen.

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