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STEM FASCIATION IN *LILIUM HENRYI* CAUSED BY NEMATODES

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SUMMARY

The spontaneous appearance of fasciation in flower stalks and stems of clones of *Lilium henryi* is not genetically determined. Fasciated plants have been shown to be free of bacterial and virus infections, and phytohormones and wounding as causal agents of fasciations can be excluded. All fasciated plants had an increased population of nematodes in the soil layers surrounding the bulbs. The nematodes were identified as being predominantly from the *Rotylenchus* and *Ditylenchus* species, as well as some others. The injection of a total extract of *Ditylenchus dipsaci* induced fasciation in 56% of the treated plants.

1. INTRODUCTION

Fasciation, a band shaped deformation of the stem, is found widespread in the plant kingdom (reviews WHITE 1948, GORTER 1965). Since the classical investigations of HUGO DE VRIES (1894, 1899a, b) spontaneous fasciations are assumed to be genetically determined. This is the case with many dicot species where true-breeding, fasciated lines have been found (GOTTSCHALK 1977; EENICK & GERRETSEN 1980; ALBERTSEN et al. 1983). However, in many asexually propagated species the origin of fasciation is unknown (BOKE & ROSS 1978). With the exception of *Narcissus* (HESLING 1968), fasciation in monocots seems to be rare (ANONYMUS 1869; HUNTLEY 1902; BAIRATHI & NATHAWAT 1978).

In the lily species, *Lilium henryi* Bak, which has been cultivated in the Botanical Garden of the University of Nijmegen for more than 25 years, fasciations have been observed in changing proportions through many years. This plant material was originally cultivated for biochemical analyses of developmental processes (e.g. LINSKENS & SCHRAUWEN 1968), thus the teratological deviations have been documented for a long time. To find out whether or not the fasciation in this species is inherited or caused by natural environmental factors, detailed analyses have been conducted from 1974.

2. MATERIAL

Four clones were reared by vegetative propagation from four equally sized, healthy looking bulbs of *Lilium henryi,* derived from a seed sample originating from the University of Cologne (FRG) in 1959.

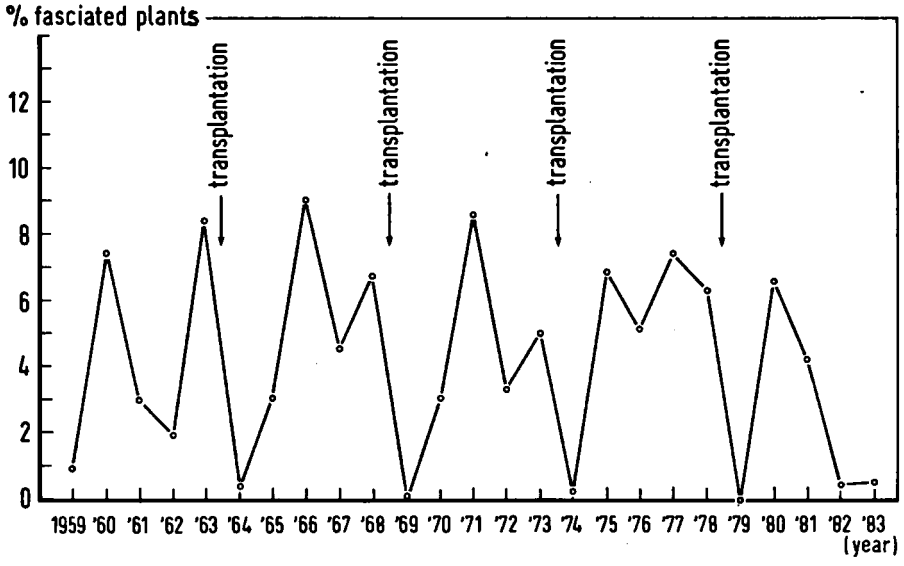

Fig. 1. Percentage of fasciated plants of *Lilium henryi* in clone 4 (Botanical Garden of the University Nijmegen). The number of plants in culture varied over the years from between 250 and 500 individuals. The bulbs were transplanted onto new beds every fifth year.

3. RESULTS

3.1. Exclusion of inheritance

In the first two years of cultivation only one clone, no. 4, showed some fascination, increasing from 1% to 7.4%.

To check if the fasciation had a genetic background, cross- and self-pollinations of fasciated plants have been carried out for many years. These breeding experiments were difficult because of the high percentage (95–100%) of female sterility of flowers from fasciated plants (flowers lacked pistils).

None of the plants originating from seeds of self- and cross-pollinated fasciated plants displayed fasciation within the first two years. From these and many other, equally negative, results it was concluded that in *Lilium henryi* the expression of fasciation is not genetically determined.

3.2. Environmental factors

Based on an extensive literature study the following experiments were carried out in order to find out if natural environmental or artificial applied factors were responsible for fasciation in *Lilium henryi*:

1) Light- and electron-microscopical investigations did not reveal any evidence of bacterial, virus or nematode infections in the leaves, bulb, scales, roots, stems, flowers, or vegetation points of fasciated plants.
2) Infection of healthy lily plants with *Corynebacterium fasciens*, which generates fasciation in many other species (THIMANN & SACHS 1966) did not cause

fasciated plants. Extracts from epiphytic bacteria isolated from fasciated plants and then applied to non-fasciated plants (TILFORD 1936) gave no fasciation.

3) The wounding of healthy looking plants in various stages of development by decapitation, defoliation, destruction of the growing points, injection of water into the stems never resulted in fasciation.
4) The application of growth hormones (IAA, gibberellins, kinetin) by injection or by means of wool fat to various organs and at different developmental stages did not result in fasciated plants.
5) Extra manure, higher temperatures, water stress also did not lead to fasciation. These experiments have been carried out also with bulbs derived from plants displaying a high degree of fasciation in the preceding year.
6) The application of pressure juice from fasciated to non-fasciated plants by means of injection, leaf application and continuous application with cotton threads had no effect on the healthy plants.
7) The grafting of fasciated stems as scions onto healthy plants, and *vice versa,* i.e. a transfer of fasciation, was not possible.
8) The planting of bulbs from normal plants into soil samples in which fasciated individuals had been grown, did not result in fasciation.
9) Vegetatively propagating plants by the rooting of stem sections did not result in fasciated descendents, even when the stocking delivering plant was fasciated.

L. henryi plants showed, independent of the fasciation phenomenon, reduction of vigour when planted on the same plot in consecutive years. As the soil was fertilized every year, the reduction of vigour was ascribed to an increase in the abundance of nematodes in the soil. As in the first season after the transfer of bulbs to a new plot no or hardly any fasciation was observed, whereas lilies grown on the same plot for more than one season every year showed a number of fasciations (*fig. 1*), the possibility was envisaged that an increased number of nematodes in the soil might be responsible for an increase in the number of fasciated plants growing in that soil. This supposition could be confirmed by experiments with marigold.

In certain years *Tagetes* plants (*Tagetes patula, T. erecta*), plants ascribed to have the ability to prevent fasciation (OOSTENBRINK et al. 1957), were planted between the rows of the lilies after the flowering period. This occurred in 1961, 1966, 1971, 1975, 1980 and in 1982. After this mixed culture of lilies and *Tagetes* a slight decrease of the number of fasciated plants occurred (*fig. 1*). The observations lead to the idea that nematodes could be responsible for the fasciation of *L. henryi.*

3.3. Nematodes as a fasciation promoting principle

To test the assumption that the abundance of nematodes is the determining soil condition responsible for the induction of fasciation in our lilies, the relation between the occurrence of nematodes and fasciation was investigated.

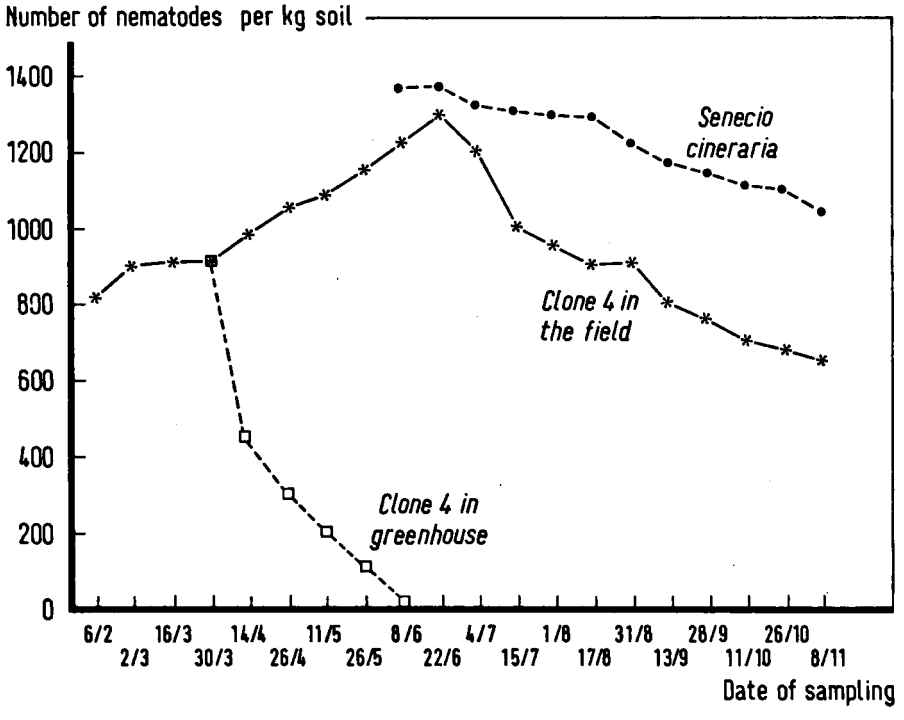

Fig. 2. Changes in the nematode population in soil samples surrounding the lily bulbs during a growing season, under field conditions (—*—*—*) and after transfer into pots and placing in the greenhouse (--□--□--□).

A method was developed to collect the nematodes quantitatively from soil samples (see appendix). In soil in which lily bulbs had been grown for four years, in March/April 1977 on an average about 950 nematodes per kg soil were found. During the growing season the number of nematodes in the soil surrounding the bulbs of fasciated plants increased from about 800 in February to about 1300 in the beginning of July and then decreased to about 650 in November (*fig. 2*) (sampling at two weeks intervals). A similar decrease in the same period was found around the roots of a fasciated plant of *Senecio cineraria*. In the soil around bulbs transferred in April to pots placed in the greenhouse the number of nematodes decreased continuously (*fig. 2*).

The nematode population consisted predominantly of the species *Ditylenchus dipsaci* (KÜHN, 1857; FILIPJEV 1936; ANONYMOUS 1972) and *Rotylenchus* spec. (*fig. 3*).

The next step was an experiment to induce fasciation by infecting clean bulbs with various amounts of nematodes isolated from soil samples surrounding fasciated plants, or from manure. Only in 5 plants from 35 grown from treated bulbs was a slight fasciation observed.

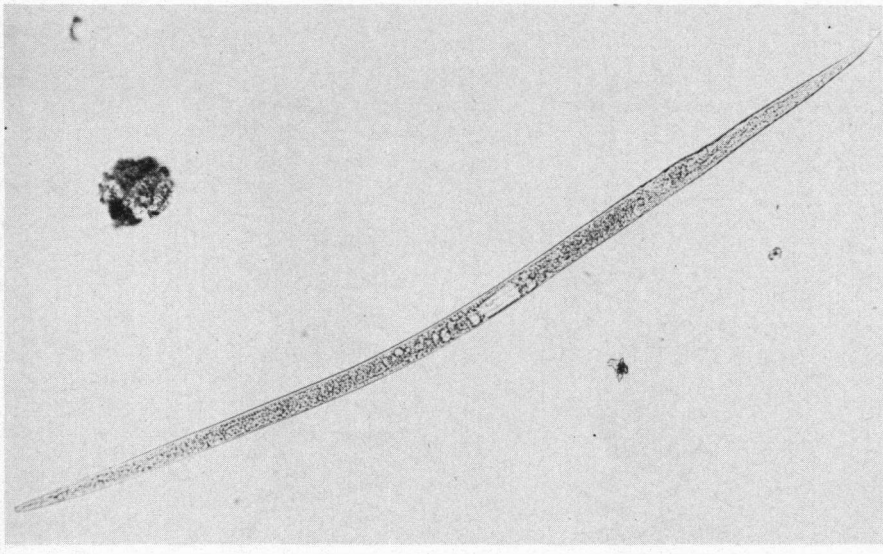

Fig. 3. The nematode from soil samples around the lily bulbs, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev 1936. Photograph: C. Stumm.

The application of water extracts containing nematodes (*D. dipsaci*) to normal plants by injection into bulbs during the months April and May did not give rise to fasciations. The bulbs remained in the soil in the field during a mild winter. In the following vegetation period, however, 56% of all treated plants (n = 20) showed fasciation (*fig. 4b*). One plant had a partial fasciation, but grew during summer into a normal plant. All control plant remained without fasciation.

This experiment provides good evidence that nematodes are responsible for fasciation in *L. henryi*.

4. DISCUSSION

From the observations and data presented here it becomes evident, that fasciation in *L. henryi,* a teratological phenomenon which results in the broadening of the main stem, multiplicity of flower parts and in increased volume and weight of the plant, is not genetically determined. In this lily species fasciation occurs in response to nematodes in the soil surrounding the bulb.

The bulbs of fasciated lily plants were always surrounded by a strongly viable nematode population. The most abundant species was *D. dipsaci,* a species which is known sometimes to inhabit bulbs and stems in many other plant species (SEINHORST 1961).

As only one of the four clones of *L. henryi* under investigation showed fasciation, the possibility of the participation of a genetical factor in the determination of the disposition for fasciation also in our lilies cannot be excluded.

Fig. 4. Fasciated plants of *Lilium henryi*.
a. experimentally induced fasciation after application of water extracts of nematodes.
b. spontaneous fasciation of lily under field conditions.

Dependent on culture conditions, the number of nematodes in the close vicinity of the bulbs can increase. However, nematodes have never been found in the tissue of the bulbs, stems or flower parts. *Ditylenchus dipsaci*, the nematode species found abundantly around *L. henryi*, normally does not enter host plants (SEINHORST 1959, 1961).

The application of total extracts of nematodes to non-fasciated plants lead to fasciation. The inducing effect of the nematodes must be an indirect one. There is some evidence that growth hormones secreted by nematodes (WEBSTER 1968; CUTLE & KRUSBERG 1968; AUDING-HALM & SCHEIBE 1968; THIMANN & SACHS 1966) and other organic substances (MYERS 1963) play a crucial role in the induction of fasciations but nematodes are able to secrete or excrete a variety of substances which also might be active in the induction of lesions and teratological structures (KRUSBERG 1963).

Indirect effects of nematodes surrounding the bulb cannot be excluded since they can function as incitants, carriers or vectors for other pathogens (PITCHER 1965; WEISCHER 1968), as e.g. viruses (WEISCHER 1969), fungi (DÜRSCHNER 1983) and bacteria (POWELL 1971).

Also, the protecting effect of mixed cultivation with *Tagetes* (OOSTENBRINK et al. 1957; MEIJNEKE & OOSTENBRINK 1958; OOSTENBRINK 1960), which prevented fasciation in clone 4 of *L. henryi* can now be explained. The root system of *Tagetes* is known to secrete nematocidial substances (UHLENBROCK & BIJLOO 1958, 1959) which suppress the nematodes. The reduction of the number of fasciations in the field in the years after mixed crop cultivation can be explained by the suppression of nematodes causing fasciation by marigold's root excretion of nematicidal substances.

The question remains: why was it never possible, neither under normal nor under experimental conditions, to obtain 100% fasciated plants among clone populations? This might be explained by the fact that for the teratologically deviated developmental process the external inducer must be present in the right concentration and at the right phase (SCHEIBE & WÖHRMANN-WILLMANN 1957) of the gene dependent reaction chain.

It can be supposed that clone 4, which in our material showed a certain percentage of fasciation regularly through the years, is genetically different from the other clones. Clone 4 can be considered as less genetically resistant to nematode interactions (SIDHU & WEBSTER 1981), but more sensitive to the morphogenetic substances secreted or excreted by the nematode population which inhabits the soils around the bulb. A certain relationship seems to exist between the size of the nematode populations and its physiological conditions. Complementary genetical systems between plants and nematodes seem to act here.

APPENDIX

Elutriator for the isolation of nematodes from soil samples: In an upwards moving water stream two forces act on a particle: the downwards directed gravitational force and an upwards directed force of convection due to the friction in the water. The motion of the particle in the medium depends on these two forces (*fig. 5*).

Principle of elutriation: In an elutriator particles are classified in an upward directed water flow according to their fall velocities.

The fall velocity w of a particle is given by:

$$w = \sqrt{\frac{2Vp\ g\ (\rho_p - \rho_w)}{C_D A_\perp \rho_w}}$$

in which

Vp = the volume of the particle (m^3)
g = gravity acceleration (m/s^2)
ρ_p = particle density (kg/m^3)
ρ_p = water density (kg/m^3)
$A_\perp$ = cross sectional particle area perpendicular to the water velocity (m^2)
C_D = drag coefficient (−)

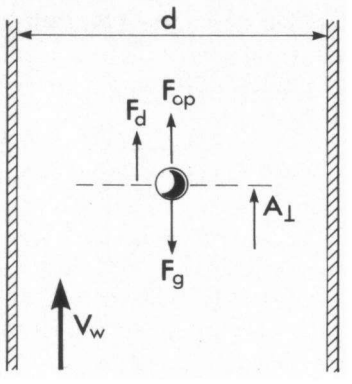

Fig. 5. Forces acting on a particle in a moving fluid
F_g = gravity force
F_{op} = buoyance force
F_d = drag force
d = column diameter
$A_\perp$ = cross sectional area of the particle perpendicular to the relative fluid velocity
V_w = water velocity

Particles with a fall velocity smaller than the water velocity applied in the elutriating column are carried upwards by the water flow and leave the elutriator at the top over-flow opening. Particles with a fall velocity larger than the water velocity are sinking down in spite of the upflowing water and end up in the heavy fraction reception vessel. The water velocity is chosen in such a way that a desired separation in top product particles and bottom product particles is achieved. This often requires an estimation of $A_\perp$ and C_D, which is difficult for non-spherical particles with no fixed orientation in the water flow. C_D may e.g. vary between the value 0.3 and 1.2 (C_D = 0.45 is a typical value for a spherical particle) depending on particle shape, orientation and density. In practice the optimum value for the water velocity is determined experimentally by analysing the separation performance of the column as function of the water velocity applied. By changing the speed of the medium stream, a separation of particles, dependent on the mass ρ_p, takes place: heavier particles move downwards, lighter particles move upwards. By interception of the lighter particles in the upper part of the column by adequate sieves, it is possible to obtain the heavier particles separated. By repeated elutrition the separation can be improved.

Application: For the isolation of nematodes a column with a diameter of 50 mm was chosen (*fig. 6*) because it can then be expected that hindering boundary layers at a streaming velocity of 0.114 l/min are negligible. Theoretically, a minimum diameter of 35 mm is necessary. The size and shape of the nematodes is variable since they tumble strongly in the water stream resulting in a variable effective area $A_\perp$. In practice, however, the assumption is made that the nematodes are spheres with a specific mass ρ_p of about 1.05×10^{-3} kg m^3. It turns out that the results under this assumption are quite reasonable.

Usage of elutriator: The soil sample with known weight, is pretreated in an appropriately sized beaker and mixed with water. After some stirring the floating fractions on the surface and the supernatant are discharged. After starting the water stream, the pretreated sample is divided into small portions and applied to the funnel (T). The light fraction in the upper part (A) of the elutriator is collected in sieve Z (mesh size 50 μm). The heavier fraction is collected in the lower part of the column (B) in a flask (O). The light fraction (from sieve Z) can be further cleaned by a repeated treatment in the elutriator, but now at a lower speed waterstream, so that then the nematode fraction results as a heavier fraction and is quantitatively collected in bottle O, from which the nematodes can be isolated using the above mentioned sieve.

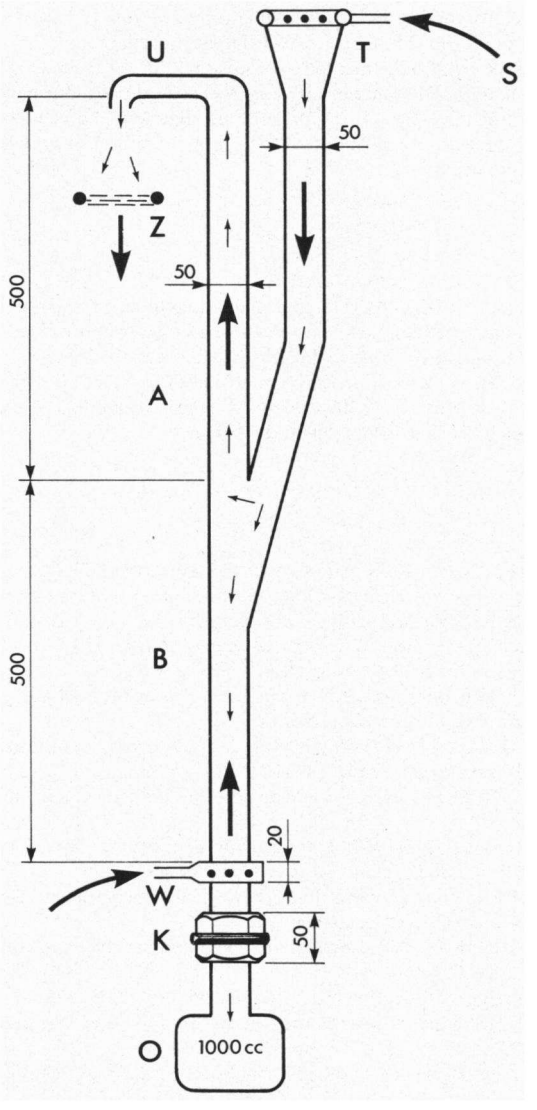

Fig. 6. Elutriator, schematic drawing (all dimensions in mm unless indicated otherwise).
➞ direction of water flow
→ direction of particle motion
A = elutriating column, classification of the light fraction
B = elutriating column, classification of the heavy fraction
S = water inlet for circular washing tube
T = supply funnel for soil sample
U = top overflow opening
Z = sieving device (mesh width 50 μm)
K = connector for the heavy fraction reception vessel
W = central water inlet for the elutriation
O = heavy fraction reception vessel

Efficiency: With the elutriator method it is possible to recover 90% of the nematodes in a given soil sample, about 5% of the individuals are lost during the procedure.

In comparison with the classical ice method by which only 20% of the individuals could be recovered, about 70% remain in the soil sample, and more than 10% are lost during the procedure. Also, the time of isolation using the elutriator is more efficient than with the ice method: for one sample of about 1000 g with the ice method about 10 to 12 hours are necessary, whereas the elutriator method needs no more than 2 hours.

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