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Mycoplasma-like organism in the rhizomes of *Cirsium arvense* L. and *Rubus idaeus* L.

Mykoplasma-lignende organismer i rhizomer hos Cirsium arvense *L. og* Rubus idaeus *L.*

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Summary
Mycoplasma-like organism (MLO) are shown by electron microscopic examination to survive during winter time in the rhizomes of *Cirsium arvense* L. and *Rubus idaeus* L. The MLO's have a different appearance but change as soon as the plant starts a new active period in spring time. The diffence in the ultrastructural appearence of the MLO's in the rhizomes during winter time as compared to the MLO's in the actively growing plants is described.

Key words: Electron microscope, MLO, *Cirsium, Rubus,* root system.

Resumé
Mykoplasma lignende organismer (MLO) overvintrer som indskrumpne organismer i rhizomerne på flerårige planter som *Cirsium arvense* L. og *Rubus idaeus* L. (agertidsel og hindbær). MLO ændrer form ved forårstide, når planten vågner op til en nye vækstperiode. Det bliver diskuteret, hvorledes MLO videreføres hos træer og enårige planter og, om forholdet gælder for andre flerårige planter.

Nøgleord: Elektronmikroskop, MLO, *Cirsium, Rubus* og rodsystem.

Introduction

This report provides new information on mycoplasma-like organism (MLO) in the rhizome system during winter time of infected plants of *C. arvense* and *R. idaeus*. Under Danish conditions this may be the way MLO survive the low winter temperatures, and it is discussed whether other plants react in the same manner.

Material and Methods

Rhizomes of *C. arvense* and *R. idaeus* were dug up in the month of August, where heavily infected plants with typical witches broom symptomes and malformed flowers were detected during the summer of the same year. The rhizomes were planted outdoors in plastic pails covered with 20 cm of soil. After a period at very low temperatu-

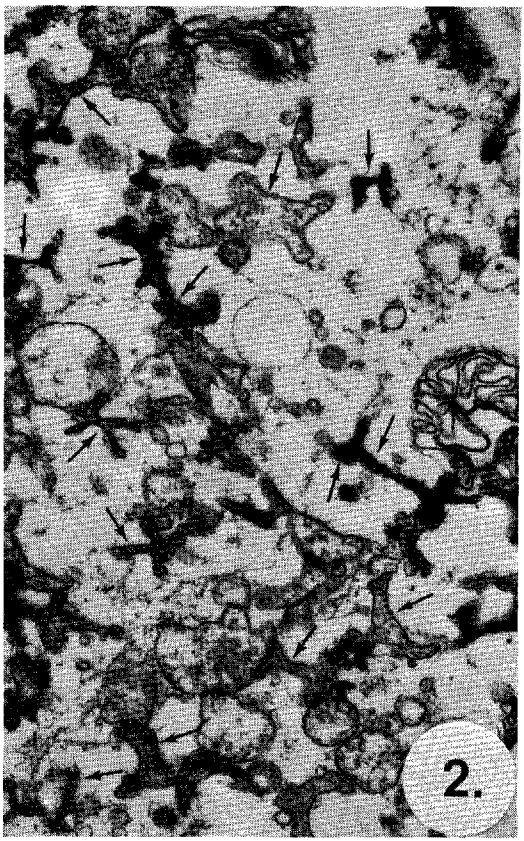

Fig. 1a. A sieve tube from *Cirsium arvense* rhizome at wintertime. The organisms are static and have a different appearance from normal active MLO's. × 19.200.

Fig. 1b. A close up of winter MLO's. The three-layered membrane is not clearly detected, and the organisms are shrunk. × 54.700.

Fig. 2. Branched MLO's from *Rubus idaeus*. The arrows show the shrunk organisms from the old rhizome. × 65.600.

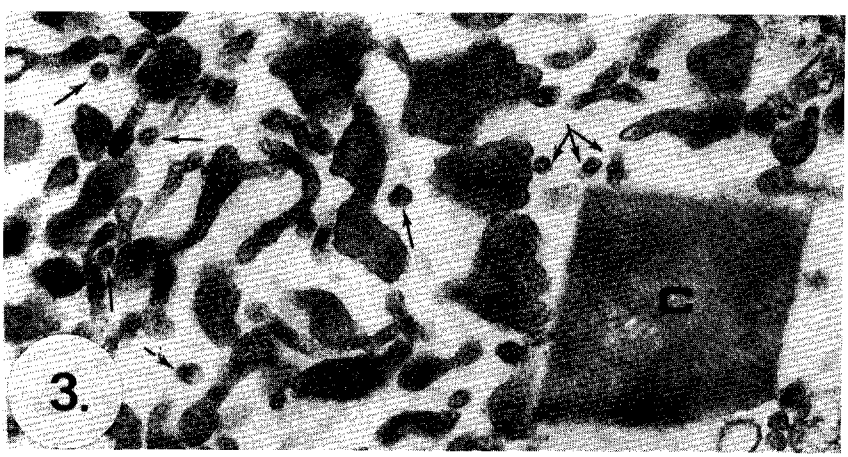

Fig. 3. Sieve tube from new root sprouts of *Cirsium arvense*. The polygonial shape has changed and the membranes are discerbed. Elementary bodies (arrows) indicate new life in the MLO population. C. = crystal. × 65.600.

res (below 20°C) the material in mid January was moved to a greenhouse with temperatures of 18–20°C and provided with artificial light.

After the rhizomes started to grow, the material was taken out for preparation for EM examination.

Tissues app. 10×0.5 mm were cut from different parts of the old rhizome and from the tip of new rhizomes. The material was fixed (*Karnowsky*, 1968) for 4 hours at 4°C. Postfixation took place in 2% osmium tetroxide (*Caulfield*, 1957) for 17 hours overnight also at 4°C. The material

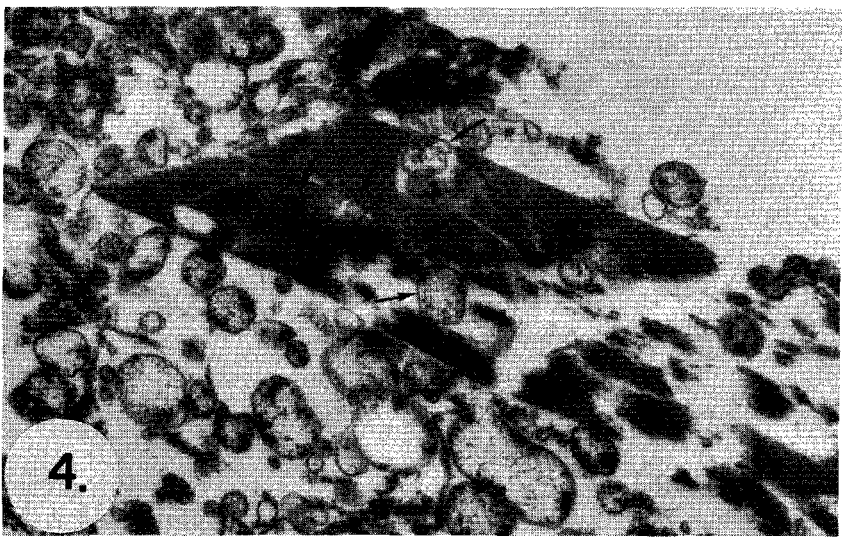

Fig. 4. Sieve tube from sprout of *Cirsium arvense*. The number of MLO's now seem quite normal. The crystal (C) shows an embedded MLO. × 25.500.

Fig. 5. A close up of embedded MLO with a clear 3-layer membrane. The MLO's are trapped in crystal formation. A lattic-work seems to hold the MLO's in a firm grip (arrows). × 102.000.

was then rinsed 4 times in distilled water, and then impregnated for 1 hour in saturated uranyl acetate and embedded in Spurr resin (*Spurr*, 1969).

Sections were cut on a LKB ultramicrotome, mounted on formvar coated grids and stained with lead citrate (*Reynolds*, 1969) and uranyl acetate in methanol, ethanol and water (1:1:3) (*Hooper & Weise*, 1972) and examined in a Philips 201 electron microscope.

Results

MLO's were detected in the phloem of the rhizomes in both *C. arvense* (Fig. 1) and *R. idaeus* (Fig. 2) but with difficulties and at a very low concentration in *R. idaeus*.

The MLO concentration was low in the individual cells, but a high number of MLO infected cells could be located. The appearence of the MLO's in the old rhizomes is different from the well known MLO seen in active growing plants. In the rhizomes of old growing parts the MLO's had an uneven, polygonale shape (Fig. 1 A and 1 B) and branched bodies appeared star-like and looked shrunken and collapsed.

In the sections from the newly formed rhizomes the number of the organisms was high, their shape was rounded, elongated and dark coloured (Fig. 3) with a destinct 3 -layered membrane. Many elementary bodies could be seen and crystal bodies are often found in association with MLO's in *C. arvense* (Fig. 3–5) but also in *R. idaeus*. The number of crystals is appearently correlated with the amount of MLO's found in the tissues.

Conclusion and discussion

The presence of MLO's in winter rhizomes in *C. arvense* and *R. idaeus* indicates the pattern of survival in the winter period. The appearance of the individial organismes in the rhizomes corresponds to the resting condition of the host plant.

Nutrients are scares during winter time and the physiological activity is lowered to a minimum. In the spring time the plant awake to a new life and a new active period. The MLO's follow this pattern and become rounder and bigger and many more elementary bodies are seen, further a higher number of organisms appear densely stained in electron microscopic preparations.

This pattern is most likely to take place in *Trifolium, Ephilobium* and *Stellaria* (*Begtrup & Thomsen*, 1975) and also in *Silene* and *Agrostemma* (*Begtrup & Lange*, 1977) and in *Helenium* (*Begtrup*, 1975) and likely other herbaceous plants.

We have no information on MLO in trees and in many other herbaceous plants. In trees a similar survival is likely with some alterations, but in many collateral perennial plants this pattern is not possible.

This reports however gives evidence of survival of MLO in the root systems of *Circium* and *Rubus* and probably in other herbaceous plants.

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Literature

Begtrup, J. (1975): Mycoplasma like organisms as pathogens in Denmark. Tidsskr. Planteavl 79, 317–325.

Begtrup, J. (1975): Mycoplasma like organisms in *Helenium sp.* Phytopath. Z. 82, 356–358.

Begtrup, J. & Thomsen, A. (1975): Mycoplasma like organisms in phloem elements of *Cirsium, Stellaria* and *Ephilobium*. Phytopath. Z. 83, 119–126.

Begtrup, J. & Lange, L. (1977): Mycoplasma like organisms in phloem elements of *Silene vulgaris* and *Agrostemma githago*. Phytopath. Z. 90, 164–171.

Caulfield, J. B. (1957): Effect of varying the vehicle for 0_s0_4 in tissue fixation. J. Biophys. and Biochem. Cyt. 3, 827–830.

Hooper, G. R. & Weise, M. W. (1972): Cytoplasmatic inclusions in wheat affected by wheat spindler streak mosaic. Virology 47, 664–672.

Karnowsky, M. J. (1965): A formaldehyd-gluteraldehyd fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27, 137.

Reynolds, E. (1963): The use of lead citrate at high pH, as electron opaque stain in electron microscopy. J. Ultrastruct. Res. 26, 31–43.

Spuur, A. R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31–43.

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