

## Host-endophyte interaction between *Lophodermium piceae* and *Picea abies*: cultural, ultrastructural and immunocytochemical studies\*

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The frequency of endophytic infections by *Lophodermium piceae* isolated from needles of *Picea abies* varied with needle age, stand density, height of the branches and sampling site. Endophytic hyphae of *L. piceae* were detected both intercellularly and intracellularly in near-surface needle tissues by scanning and transmission electron microscopy. In tissue sections hyphae were identified by immunoelectron microscopy. Infection loci were limited to very small tissue areas. The penetration of plant cell walls by *L. piceae* hyphae was accomplished by forming a very thin hyphal tip. Endophytic hyphae were observed to form endocells, and were surrounded by a capsule-like sheath. Papilla-like appositions were observed on cell walls in the mesophyll of infected needles.

Little is known on the ecology of endophytes and related host-fungus interactions (CARROLL, 1986). Furthermore only few reports available deal with the direct light or electron microscopic observation of endophytic hyphae within their host tissues (SUSKE & ACKER, 1987, 1989a, 1989b; STONE, 1987, 1988; SIEGEL & al., 1987; HINTON & BACON, 1985; CROMEY & COLE, 1984; FINERAN & al., 1983; BERNSTEIN & CARROLL, 1977).

This paper outlines briefly the work done in our laboratory over the last 5 years on fungal endophytes by combining cultural studies with ultrastructural and immunocytochemical observations.

### Materials and methods

Endophytic fungi were isolated on malt agar from surface-sterilized Norway spruce needles (*Picea abies* KARST.; RACK & BUTIN, 1984) collected in the Fichtelgebirge (Northeast Bavaria, Federal Republic of Germany). From 1985 to 1989 samples were taken from light and dense spruce stands at a declining site (Oberwarmensteinach) and an

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apparently healthy site (Wülfersreuth). For studies on multiple infections in individual needles, surface-sterilized needles were sectioned in 0.4–0.7 mm long segments. The segments were incubated in serial order on malt agar. Each series of contiguous segments infected by *Lophodermium piceae* (FUCK.) HÖHN. was scored as a single infection, termed a run of infection (BERNSTEIN & CARROLL, 1977).

Physiological tests were carried out to assess the capability of *L. piceae* to metabolize complex substrates within spruce needles (HANKIN & ANAGNOSTAKIS, 1975; TAYLOR, 1974; SUNDMAN & NÄSE, 1971; RAUTELA & COWLING, 1966; KÄÄRIK, 1965; FERNLEY, 1963; SIERRA, 1957).

For scanning and transmission electron microscopy (SEM and TEM) investigations needle samples were selected and prepared as described previously (SUSKE & ACKER, 1987, 1989a).

*L. piceae* specific immunoserum was obtained by absorbing a rabbit anti-*L. piceae* immunoserum with different fungal antigens. The obtained *L. piceae* specific immunoserum was applied in combination with the immunogold-technique (ACKER, 1988) for the identification of endophytic *L. piceae* hyphae in situ in thin sections of infected spruce needles (SUSKE & ACKER, 1989b).

## Results and discussion

### Cultural investigations

*L. piceae* was clearly the major endophytic colonizer of asymptomatic Norway spruce needles. The infection frequency by this fungus in foliage taken from 4–6 m high branches of trees at the declining site (Oberwarmersteinach) increased with the needle age from ca. 1% in the youngest needles to ca. 30% in 4- to 5-year old needles. Needles taken from a dense stand were more frequently infected (ca. 50–90%). Similar patterns of infection were reported for many other endophytes (e.g. STONE, 1987, 1988; PETRINI & al., 1982; PETRINI & CARROLL, 1981; MILLAR & RICHARDS, 1975). Lower rates of infection by *L. piceae* (1–5%) were determined for needle samples taken from the apparently healthy site at Wülfersreuth.

Needles from declining and apparently healthy trees at Oberwarmersteinach revealed similar infection frequencies by *L. piceae*. This observation indicates that unknown stand factors affect the incidence of *L. piceae* more than the state of health of spruce trees, as already reported by BARKLUND (1987).

The highest infection rates by *L. piceae* (96%) were recorded for asymptomatic, 3- to 5-year old needles sampled from 2 m high branches at Oberwarmersteinach. This observation is in agreement with the assumption of BUTIN (1986) that the incidence of fungal

endophytes in spruce needles decreases with increasing height of the canopy.

The extent of needle colonization by a single individual is important to understand the interaction between endophytes and their hosts. Our investigations revealed that 50% of the examined needles taken from light spruce stands had a single isolated run of infection by *L. piceae*. Nearly 90% of these runs of infection extended over only one needle segment of 0.4 to 0.7 mm length. The remaining portion of these needles was colonized by two or three individual infections. On the other hand 2 to 5 separate runs of infection by *L. piceae* were isolated from 92% of the asymptomatic needles collected from dense spruce stands. More than 50% of these runs of infections extended over 2 to 9 needle segments. These observations suggest that the infections by *L. piceae* in green needles remain restricted to small tissue areas. The longer runs of infection determined for needles taken from dense stands suggest that either the hyphae had begun to spread further in the needles or a large number of individuals produced separate infections that could not be distinguished by the cultural technique applied (STONE, 1986).

Substrate utilization tests demonstrated that *L. piceae* possesses a broad range of enzymatic activities (e.g. cellulase, pectinase, xylanase, lignase, and cutinase) which can be associated with leaf penetration and long-term residence within leaves.

#### Electron microscopy (SEM and TEM)

Intercellular and intracellular *L. piceae* hyphae in symptomless needles were localized by scanning and transmission electron microscopy. Endophytic infections were observed to consist of single hyphae or very limited mycelia colonizing near-surface needle tissues. In agreement with our cultural investigations this provided evidence that endophytic infections by *L. piceae* remain restricted to small tissue areas, and that the sparsely distributed hyphae may cause only little damage. This may explain why infections by *L. piceae* remain latent and asymptomatic during the endophytic phase of the fungus.

The penetration of plant cell walls by *L. piceae* hyphae was accomplished by a very thin hyphal tip (ca. 0.5  $\mu\text{m}$  in diameter). Intercellular and intracellular swellings were observed along endophytic hyphae colonizing near-surface needle mesophyll (SUSKE & ACKER, 1987). In thin sections these swellings were identified as endocell containing hyphae (Fig. 1) or clusters of hyphal cells forming irregular shaped complexes (SUSKE & ACKER, 1989a). Most researchers agree that the formation of intrahyphal hyphae (endocells) is a result of abnormal or stressful conditions, either from some

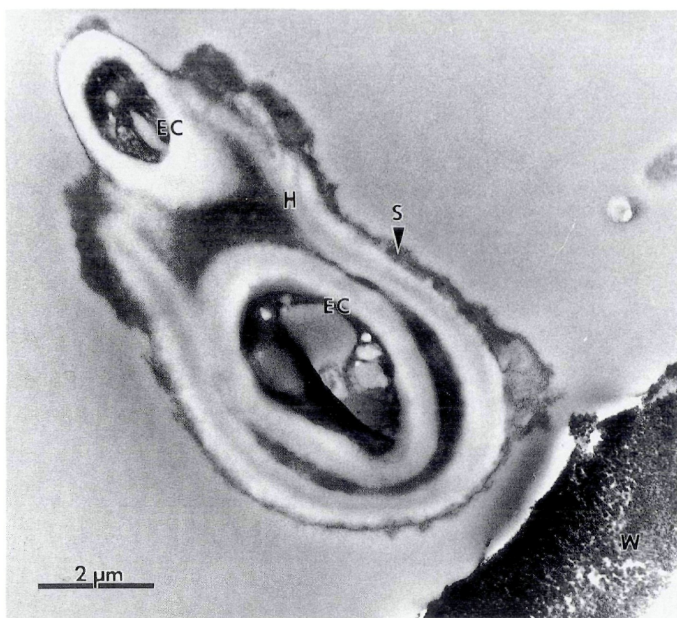


Fig. 1. – Intercellular *L. piceae* hypha (H) containing two endocells (EC). The upper endocell seems to penetrate through a gap in the cell wall of the enclosing hypha. Mesophyll cell wall (W), hyphal sheath (S).

deleterious action on fungal cells or very slow fungal growth (BENHAMOU & OUELLETTE, 1986; PENDLAND, 1981). The observed endocells within endophytic *L. piceae* hyphae probably represent resistance structures formed by the fungus to survive within the tissues of living needles. Endophytic *L. piceae* hyphae were surrounded by a capsule-like structure or sheath of unknown chemical composition and function. Further studies are needed to clarify its role in adhesion to sister hyphae and plant tissues, as well as its biological function, such as involvement in nutrient absorption, and protection against desiccation or against toxic host components (BENHAMOU & OUELLETTE, 1986; HINTON & BACON, 1985).

The infection of green needles by *L. piceae* resulted in the formation of papilla-like appositions on mesophyll cell walls (Fig. 2). Such wall appositions were not observed in uninfected green needles. Papilla-like structures were described as protective structures of plant cells established as response to fungal infections (RIDE, 1978;

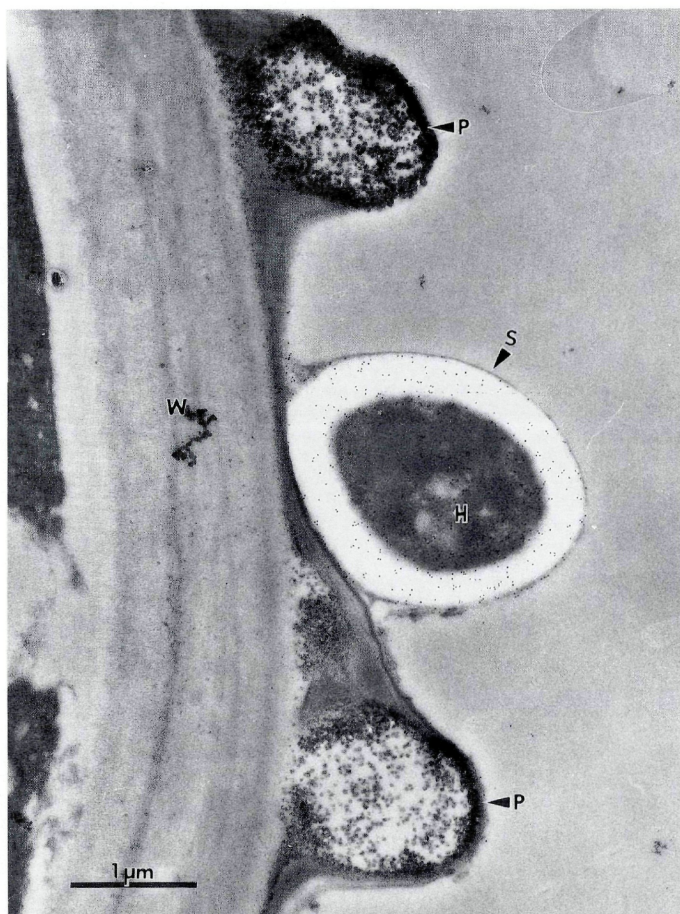


Fig. 2. – Intercellular hypha (H) after immunogold-labeling. The labeling of the hypha (dark dots) demonstrates that it belongs to *L. piceae*. Papilla-like wall appositions (P) can be seen on the mesophyll cell wall (W). Fungal sheath (S). Micrograph from SUSKE & ACKER (1989b).

AIST, 1976). Therefore, the structures observed in spruce needles may represent defence structures established to limit endophytic infections by *L. piceae*.

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