Silva Gabreta	vol. 3	p. 7–16	Vimperk, 1999

Armillaria populations and pathology at different forest sites of South Bohemia

Populace rodu *Armillaria* a jeho patologie na rozdílných lesních stanovištích jižních Čech

Jiří Marek [1] & Anna Lepšová[1,2]

[1] *Faculty of Biology, University of South Bohemia, Na sádkách 7, CZ-370 05 České Budějovice*
[2] *Institute of Landscape Ecology, Czech Academy of Sciences, Na sádkách 7, CZ-370 05 České Budějovice*

Abstract
The occurrence of *Armillaria* species in Šumava and Novohradské hory Mts. was investigated during 1993. The species composition was identified both morphologically and by interfertility testing using diplo-haplo pairing tests. To determine the size of individual genets test of somatic compatibility was employed. We confirmed *Armillaria ostoyae* in about 80 years old Norway spruce plantation near Stožec, Šumava Mts. which was present as carpophores and in lower stem rot. One large genet was detected in the permanent plot at the site of massive tree breaks. Saprotrophic species *A. bulbosa* and *A. cepistipes* were involved in dead conifer wood decomposition. Pathogenic *Armillaria ostoyae* and saprotrophic *A. cepistipes* occurred on the plot in old grown beech forest Žofinský prales, Novohradské hory Mts. on dead wood of *Abies alba* and also on dead wood of *Fagus sylvatica*. No *Armillaria* species was found in spruce forest type at 1300 m elevation in Šumava Mts. The results suggest that *Armillaria ostoyae* is an important factor supporting the natural tree species composition for a particular environment in long term perspective.

Key words: *Armillaria*, root rot, Šumava Mts.

Introduction

Armillaria is an intensively studied genus among the plant pathogens and the epidemiology of the *Armillaria* species remains unsufficiently known (Guillaumin et al. 1994). The description of *Armillaria* populations within the ecosystem is one of the basements to contribute to the knowledge on its epidemiology. The method consists in species identification and genets delimitation of *Armillaria* within plots under the study.

Species collected as carpophores are distingushable for experienced mycologist. Some troubles may arise in delimitation *A. cepistipes* and *A. bulbosa*. Moreover, collections obtained from rhizomorphs and infected wood isolates have been identified using culture characteristics. As the progress continues identification has been based on (e.g.): the morphology of rhizomorphs on defined media (Rishbeth 1986, Jankovský 1997); interfetility testing (Korhonen 1978); serological techniques and isoenzyme pattern determination (as reviwed by Guillaumin & al. 1993; and Schulze & al. 1995). Molecular techniques are beeing employed recently (Guillaumin & al. 1994). Schulze & al. (1995) distinguished all five european annulate *Armillaria* species by restriction fragment lenght polymorphisms of ribosomal DNA (RFLPs rDNA). *Armillaria bulbosa* and *A. cepistipes* were revealed to be closely relative with interspecific similarity of 42 %. Similarity is continnually decreasing *to A. borealis* (22%), *A. ostoyae* (17%) and *A. mellea* (11%).

Pathogenicity of *Armillaria* species

Some of *Amillaria* species are known to cause a significant economic loss in forest trees and in nursery plantations as well. The pathogenicity of *Armillaria* species in Europe has been characterised roughly (GUILLAUMIN & al. 1993, 1994) and seems to be different in various areas.

Armillaria ostoyae acts as a primary and opportunistic parasite, as a casual agent of heart rot and as a saprophyte as well. As an opportunistic species it attacked *Abies alba* weakend by drought series in 1980. As a saprophyte *A. ostoyae* was found on both conifer and hardwood stumps. The subterranean rhizomorphs *of A. ostoyae* are formed abundantly and act as infective organ (MORRISON 1982, MOHAMED 1987 sec. GUILLAUMIN & al. 1993). The pathogenicity tests of species towards three years old Norway spruce and Scots pine reveales the decreasing infectivity as follows: *A. ostoyae* > *mellea* > *borealis* > *bulbosa* (SIEPMANN & LIEBIGER 1989).

Armillaria mellea is more highly pathogenic species in hardwoods and orchard trees and less pathogenic in *Abietaceae* conifers than *A. ostoyae* (RISHBETH 1982, GUILLAUMIN AND LUNG 1985 and MOHAMED 1987, sec. GUILLAUMIN & al. 1993). Susceptibility of *Abietaceae* decreases rapidly as age increases as shown by RISHBETH (1982). This species exists mostly as a primary pathogen and may persist as an opportunists as well. The growth of *A. mellea* rhizomorphs is significantly limited in soil, they can not act as organs of dissemination and preservation. They can, however be effective as infective organs provided the target roots are close enough to the substrate from which the rhizomorphs are growing.

Armillaria borealis is described as a week pathogen in western Europe and occurs on birch and cherries. The most widespread this species is in northern Europe, behaving like a week, secondary pathogen, causing but rot of *Picea abies* (KORHONEN unpubl., sec. ROLL-HANSEN 1985). In Scotland it has been characterised as a primary parasite as well as an opportunist (GUILLAUMIN & al. 1993).

Both *A. bulbosa* and *A. cepistipes* are considered as efficient decomposers of dead wood in harwood and conifer forests. Both species develop long, thick, monopodially branched , robust and perennial rhizomorphs. These organs are well adapted for finding and invading stumps and buried dead wood. Artificial trials confirmed, that *A. bulbosa* is a weaker pathogen than *A. ostoyae* and *A. mellea* (RISHBETH 1985 and MOHAMED 1987, sec. GUILLAUMIN & al. 1993). GUILLAUMIN & al. (1993) conclude that the pathogenicity of *A.cepistipes* is low and even lower than that of *A. bulbosa*.

Armillaria species in Central Europe

The occurrence of *Armillaria* species in spruce and beech forests documented SIEPMANN 1985 and SIEPMANN & LIEBIGER (1989). They found *A. bulbosa* and *A. ostoyae* in old spruce and beech forests (about 80 years) and *A. bulbosa* and *A. borealis* in 18 years old mixed forest of spruce and mapple, forest types were closed each to another. *A. bulbosa* occurred in an old beech stand, *A. cepistipes*, *A. ostoyae* and *A. bulbosa* in young spruce stand surrounded by old beech stand and *A. ostoyae* and *A. borealis* in spruce roots of an old spruce stand.

All of the five species of annulate *Armillaria* were documented in the Czech republic (ANTONÍN 1986, 1988 , 1990; GUILLAUMIN & al. 1993, JANKOVSKÝ 1997).

The ecology of *Armillaria mellea* s. str. in the Czech republic was partly elucidated by ANTONÍN (1986). The consistent information on other species has stayed unpublished yet (JANKOVKÝ 1997).

Our attempt was to confirm and evaluate the *Armillaria* species in the naturally grown old forest sites of the two mountain areas: Novohradské hory Mts. with a typical mountain beech forest; and Šumava Mts. with a naturally grown spruce forests, both areas in the South Bohemia. To compare the phytopathological behaviour, *Armillaria* species were also investigated in the first generation of a spruce forest grown after the original mountain beech canopy in Šumava Mts.

Material and methods

Study sites

Altogether five localities were searched for the occurrence of *Armillaria* species (Table 1):

Novohradské hory Mts.

(I) Žofín, Nature reserve (735-825 m elevation), alltogether 8 different forest types distinguished within the locality, some types are of a great value, representing the natural growth of mountain mixed beech forest. About 5 types were searched for *Armillaria* species:

(Ia) Mixed beech forest (750 m elevation), *Fagus sylvatica* (83.6 %), *Picea abies* (15 %) and *Abies alba* (1.4 %), old grown forest (altogether 32 trees on the plot),

(Ib) Spruce forest stand (820 m elevation), 60 years old, spontaneously invaded spruce from the surrounding man grown plantations,

(Ic) Transect through nature reserve (line transect from the point 48°39' 50" north latitude and 14° 42' 15" east longitude to the 48° 39' 47" n.l. and 14° 42' 47" e.l., 750-820 m elevation), includes 5 forest types of the reserve, alltogether *Fagus sylvatica* (87%), *Pice abies* (11.5%), *Abies alba* (1.5%),

(Id) Mixed beech forest (750 m elevation), *Fagus sylvatica* (75 %), *Picea abies* (21.9%) and *Abies alba* (3.12 %), old grown forest (altogether 140 trees on the plot).

Šumava Mts.

(II) Stožec, man grown spruce forest of the 1st generation after the original mixed mountain beech forest (48° 52' 15" n.l. and 13° 51' 00" e.l., 800 m elevation), *Picea abies* (100%), with *Calamagrostis villosa* in the understory.

Three localities were chosen in nearly naturally grown spruce forest sites:

(III) Boubín Mt. (48° 59' 40" n.l. and 13° 49' 20" e.l. and 48° 59' 20" n.l. and 13° 48' 50"

Table 1. – Sampling of *Armillaria* species, numbers of carpophores per plot, number of tissue isolates from carpophores and wooden cores.

	plot size	Control for carpophores	sample n coll./ n isol.	wood cores sampling	trees with rot found n (%) / n isol.
Movohradské hory Mts.					
Žofínský prales					
Ia beech	30 x 30 m²	14 Oct. 1993	7/5	no	no
Ib spruce	30 x 30 m²	not found		22 Jan. 1993	9 (27%)/ 0
Ic transect	10 x 800 m²	9 Oct. 1993	37/18	no	no
Id beech	60 x 60 m²	22 Sep. 1994	104/29	no	no
Šumava Mts.					
II Stožec	60 x 60 m²	2 Oct. 1993	103/88	17 Sep. 1993	50 (45%)/ 7
III Boubín	3 plots 50 x 50 m²	Sep. Oct. 1993-4	0	no	
IV Alpa	30 x 30 m²	11 Sep. &13 Oct. 1993	0	11 Sep. 1993	8 (30%)/ 0
V Trojmezí	50 x 50 m²	Oct. 1993 – 1994	0	24 Apr. 1993	7 (20%)/ 0

e.l., 1300 m elevation) *Calamagrostio villosae-Piceetum* (Tüxen 1937) Hartmann 1953, *Picea abies* 100%,

(IV) Alpa Mt. (48° 44' 46" n.l. and 13° 55' 32" e.l., 1280 m elevation) *Athyrio alpestris-Piceetum* Hartman 1959, *Picea abies* 100%,

(V) Trojmezí (48° 46' 20" n.l. and 13° 51' 00" e.l.1345 m elevation) *Athyrio alpestris-Piceetum* Hartman 1959, *Picea abies* 100%,

Sampling of fungi

Carpophores of *Armillaria* were noted throughout the whole research plots. The position of carpophores in relation to the substrate and substrate type were documented.

The carpophore sample represented a group of carpophores and consisted of one or more carpophores collected at least 1 m apart from the next one. The carpophore sample was used for species identification and for tissue isolation.

Identification of *Armillaria* species

Carpophore samples were described and stored dry for next examination. The criteria for identification were used as recomended TERMOSHUIZEN & ARNOLDS (1987) for European species and as used ANTONÍN (1986, 1988, 1990) for local populations.

Isolation and cultivation of *Armillaria* samples

The tissue for *Armillaria* isolation was obtained either from the part of carpophore cap and stem connection or from wooden cores cutted from the tree by Pressler increament corer. The latter samples were taken both in the stump and breath height as recomended WAHLSTRÖM (1992).

The MEA (1.5%) was used to isolate tissue cultures derived from carpophore and to maintain them for further testing. The Hagem's agar medium with the addition of Benomyl to exclude micromycetes was used to isolate *Armillaria* species from wooden cores (e.g. WAHLSTRÖM 1992).

The *Armillaria* genet delimitation

To distinguish the genets the tests of somatic incompatibility were employed, as described by KORHONEN (1978), ANDERSON & ULRICH (1979) and GUILLAUMIN & al. (1991). The black line between diploid isolates of different species was added as the other criterion (ADAMS 1974, RISHBETH 1982 and WAHLSTRÖM 1992).

The *Armillaria* isolates identification

Pairing of diploid isolates with haploid testers (KORHONEN 1978, GUILLAUMIN & al. 1991, WAHLSTRÖM 1992) was used to determine species of *Armillaria* in cultures. The changes of haploid mycelia from arial fluffy appearance to diploid more or less crustose mycelial growth were claimed to be a positive criterion for interfertility among isolates.

The collection of haploid tester were kindly provided by dr. K. Wahlström (Swedish University of Agricultural Sciences, Uppsala, Sweden, in 1993). Tester strains are listed as follows:

Armillaria bulbosa (Barla) Romagn., syn.: *A. gallica* Marxm.et Romagn.:
861024.1.1/1; E5: 801013.1.1/1; B4: 771128.1.1/7
Armillaria mellea (Vahl:Fr.) Kumm.:
871007.2.3/3; D3: 771031.2.1/8; D2: 771101.2.2/6
Armillaria ostoyae Romagn. Herink, syn.: *A. obscura* (Secretan) Herink:
870923.4.3/1; C6: 801028.1.1/4; C1: 770922.1.2/2
Armillaria cepistipes Velen.:

870912.1.1/1; B2: 741105.2.3/1
Armillaria borealis Marxm.et Korh.:
870908.1.2/1; 800921.1.1/4; 870831.1.1/6

Results

Žofínský prales, Novohradské hory Mts.

We found only 7 *Armillaria* carpophore samples at the locality **Ia** in 1993 and we succeeded in tissue isolation from 5 samples. All samples were identified as *A. ostoyae.* The transect **Ic** yielded 37 *Armillaria* carpophore samples, consisting of *Armillaria ostoyae* and *A. bulbosa-cepistipes* complex. 18 tissue samles were isolated. The new plot **Id** was established in 1994 nearby and 104 *Armillaria* carpophore samples were found and 29 tissue isolates were obtained. Two species were recognised, *A. ostoyae* and *A. bulbosa-cepistipes* complex (Table 1 and 2).

The presence of stem rot was tested only at plot **Ib** spruce. We found 27% of stem rot, but no *Armillaria* isolates were obtained (Table 1, last column). Trees were infected by *Heterobasidion annosus*, which was proved by tissue isolates. We did not searched for stem rot at other plots (no permition from the authorities was obtained).

Using the method of somatic compatibility we determined 6 genets of *Armillaria* complex on the plot **Id**. Determination of genets was proved by diplo-haplo pairing of representative isolates with tester strains. We confirmed 6 genets of *Armillaria cepistipes* and 1 genet of *A. ostoyae* (Fig. 3)

Šumava Mts.

The *Armillaria* occurred only at the locality **II** Stožec. We identified *Armillaria ostoyae, A. bulbosa* and *A. cepistipes.* We collected 103 carpophore samples and obtained 88 tissue isolates of carpophores (Table 1,2, Fig. 1).

All trees were searched for the lower stem rot and 50 (45%) of them were positive. The *Armillaria* isolates were confirmed in 7 samples (Table 1, Fig. 1,2). We did not find any *Heterobasidion annosus* among isolates from rotten wood.

We determined 4 *Armillaria* genets on this plot. Diplo-haplo pairing tests proved 1 genet of *Armillaria ostoyae*, 2 genets of *A. bulbosa* and 1 genet of *A. cepistipes.* Only a part of *Armillaria ostoyae* genet was recorded. It covers about 50% of permanent plot and evidently continues out of permanent plot (Fig. 2). The fungal rot of spruce stems was rather frequent with 45% infected trees. We isolated 7 *Armillaria* tissue isolates from rotten wood, which were proved to be identical with the genet of *Armillaria ostoye* on this plot (Fig. 2).

There were no carpophores, rhizomorphs nor *Armillaria* isolates in rottend wood at Boubín, Alpa and Trojmezí plots in Šumava Mts. (Table 1).

Table 2. – Species identification of *Armillaria* carpophores according to macroscopic features and tissue isolates.

	Armillaria ostoyae N / %	*A. bulbosa & A. cepistipes* N / %	Not identified N / %
Ia beech 1993	7 / 100	0	0
Ic transect	6 / 16.2	17 / 46	14 / 37.8
Id beech 1994	5 / 4.8	97 / 93.3	2 / 1.9
II Stožec	38 / 36.9	58 / 56.3	7 / 6.8

Discussion

The presence of two or three *Armillaria* species in Žofín and Stožec, respectively, reflects the similarity with Central European findings of SIEPMANN (1985) and SIEPMANN & LIEBIGER (1989), but we did not find *Armillaria borealis* at our plots. We have recorded this species only near Modrava (Šumava Mts.) until now, groving on dead *Sorbus aucuparia* in 1050 m elevation. The occurrence of *A. mellea* in the area investigated is less probable, because it is typical for lower elevations and hardwood trees (JANKOVSKÝ 1997).

Concerning the pathogenicity, we confirmed *Armillaria ostoyae* in lower stem rots of living Norway spruce trees at Stožec plot. It spreads among suitable host trees for many years and forms the large genet. Similar continual spreading was confirmed for *A. bulbosa* by SMITH & al. (1992). The other species *A. bulbosa* and *A. cepistipes* grew only on dead wood of Norway spruce at Stožec plot. The lower stem rot in Norway spruce at Žofín plot was evaluated, but no *Armillaria* was found in isolates. *A. ostoyae* grew on dead stump and standing stems of *Abies alba* and on dead wood of *Fagus sylvatica. A. cepistipes* grew on dead wood of *Abies alba* and beech.

These findings confirm the pathogenicity of *Armillaria ostoyae* on Stožec plot, where it causes the root and lower stem rot of *Picea abies*. The forest canopy is damaged by wind breakes and the forest become desintegrated. This species maybe had caused the death of *Abies alba* at Žofín plot by the infection of phloem and sapwood, but it does not cause the serious damage in the canopy with prevailing beech. *Armillaria ostoyae* may be the factor controlling the forest canopy composition in the Žofín stand conditions.

Our observation suggested, that *Armillaria* species occur at plots of both areas in beech and beech-spruce forest type. We did not confirmed the *Armillaria* species in mountain spruce forest-type of higher elevation. The size of individual genets illustrates their long-lasting occurrence, which depends on the species composition and the stand age and supports the idea of vegetative spreading by means of mycelia and rhizomorphs than by spores.

Conclusion

The occurrence of *Armillaria* was documented in forests of 750 – 800 m elevation, which corresponds to beech forest type. There were more *Armillaria* species found within one locality and of different in pathogenicity. *Armillaria ostoyae* inhabited rot in lower stem parts and roots. The large *Armillaria ostoyae* patch was identified as one individuum at Stožec plot (Šumava Mts.) and covered about 50 % of the plot. This suggests that *A. ostoyae* spreads vegetatively by means of mycelia and rhizomorphs. Continuum of suitable host tree (Norvay spruce in this case) supports the successful spreading and longevity of individual mycelium.

Dead wood material of Norway spruce and beech was colonized by *Armillaria cepistipes* at Žofín and Stožec plots. *Armillaria bulbosa* also occupied dead conifer wood like a saprophyte but only at Stožec plot.

We did not find any *Armillaria mellea* and *A. borealis* at permanent plots although we have the record of the letter species near Modrava.

There was no *Armillaria* infection and carpophores found at study plots in higher elevation (about 1300 m) in the area of spruce forest type.

Acnowledgemens. This research was supported by the Ministry of Education, Youth and Physical Education by the grants no. ***RV 339/93*** and ***339/94.***

References

ADAMS D.S., 1974: Identification of clones of *Armillaria mellea* in young–growth Ponderosa pine. *Northwest Science, 48: 21–29.*

ANDERSON J.B. & ULRICH R.C., 1979: Biological species in of *Armillaria mellea* in North America. *Mycologia, 71: 402–414.*

ANTONÍN V., 1986: Studies in annulate species of the genus *Armillaria*. I. Study of the type–specimens of *Armillaria cepaestipes* Velenovský. *Česká Mykologie, 40: 38–40.*

ANTONÍN V., 1988: Studies on annulate species of the genus *Armillaria*. II. Ecology and geographic distribution of *Armillaria mellea* (Vahl:Fr.)Kummer s.str. in Czechoslovakia. *Acta Musei Moraviae, Sci. Nat., 73: 115–121.*

ANTONÍN V., 1990: Studies on annulate species of the genus *Armillaria*. III. Species described by Josef Velenovský. *Acta Musei Moraviae, Sci. Nat, 75: 129–132.*

GUILLAUMIN J.–J., ANDERSON J.B. & KORHONEN K., 1991: Life cycle, interfertility, and biological species. *In: Armillaria root disease, Shaw C.G. & Kile G.A. (eds.), Washington, D.C, pp. 10–20.*

GUILLAUMIN J.–J. & LUNG B., 1985: Etude de la specialisation d'*Armillaria mellea* (Vahl.)Kumm. Et *Armillaria obscura* (Sécr.) Herink en phase saprophytique et en phase parasitaire. *Eur.J.For.Pathol., 15: 342–349.*

GUILLAUMIN J.–J., C. MOHAMED, N. ANSELMI, R. COURTECUISSE, S.C. GREGORY, O. HOLDENRIEDER, M. INTINI, B. LUNG, H. MARXMULLER, D. MORRISON, J. RISHBETH, A.J. TERMOSHUIZEN, A. TIRRO & B. VAN DAM, 1993: Geographical distribution and ecology of *Armillaria* species in western Europe. *Eur. J. For. Path., 23 (6–7): 321–341.*

GULLAUMIN J.–J., ANDERSON J.B., LEGRAND P. & GHAHARI S., 1994: Use of different methods for mapping the clones of *Armillaria* spp. in four forests of central France. *In: Proc. Eight Internat. Conference Root Butt Rots, Johansson M. & Stenlid J. (eds.), Uppsala 1994, pp.437–458.*

JANKOVSKÝ L., 1997: Biologie václavek [Biology of *Armillaria* species]. *Ms. PhD thesis, Les. Dřev.Fak. MU, Brno. (in Czech)*

KORHONEN K., 1978: Interfertility and clonal size in the *Armillaria mellea* complex. *Karstenia, 18: 31–42.*

MORRISON D.J., 1982: Variation among British isolates of *Armillaria mellea*. *Trans. Br. Mycol. Soc., 78(3): 459–464.*

RIHBETH J., 1982: Species of *Armillaria* in southern England. *Plant Pathol. 31: 9–17.*

RISBETH J., 1985: Infection cycle of Armillaria and host response. *Eur. J. For. Pathol., 15: 332–341.*

RISHBETH J., 1986: Some characteristics of English *Armillaria* species in culture. *Trans. Br. Mycol. Soc., 85 (2): 213–218.*

ROLL–HANSEN F., 1985, The *Armillaria* species in Europe. *Eur. J. For. Path., 15 (1): 22–31.*

SCHULZE S., BAHNWEG G., TESCHE M. & SANDERMANN H., Jr., 1995: Identification of European *Armillaria* species by restriction fragment lenght polymorphisms of ribosomal DNA. *Eur. J. For. Path., 15 (4): 214–223.*

SIEPMANN R., 1985: Über das Vorkommen von *Armillaria* – Arten und – Klonen in benachbarten Koniferenbestanden, Mischbestanden und in Laubwald. *Eur. J. For. Path.*, 15(2): 71–80.

SIEPMANN R. & LIEBIGER M., 1991: Über die Wirtsspezialisierung von *Armillaria* Arten. *Eur. J. For. Path., 19 (5–6): 334–342.*

SMITH M.L., J.N. BRUHN & ANDERSON J.B., 1992: The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature, 356: 428–431.*

TERMOSHUIZEN A. & ARNOLDS E., 1987: On the nomenclature of the European species of the *Armillaria mellea* group. *Mycotaxon, 30: 101–116.*

Wahlström K., 1992: Infection biology of *Armillaria* species*: in vitro* pectinolytic activity, infection strategy, field distribution and host responses. *PhD Thesis, Univ. Agric. Sciences, Uppsala, Sweden.*

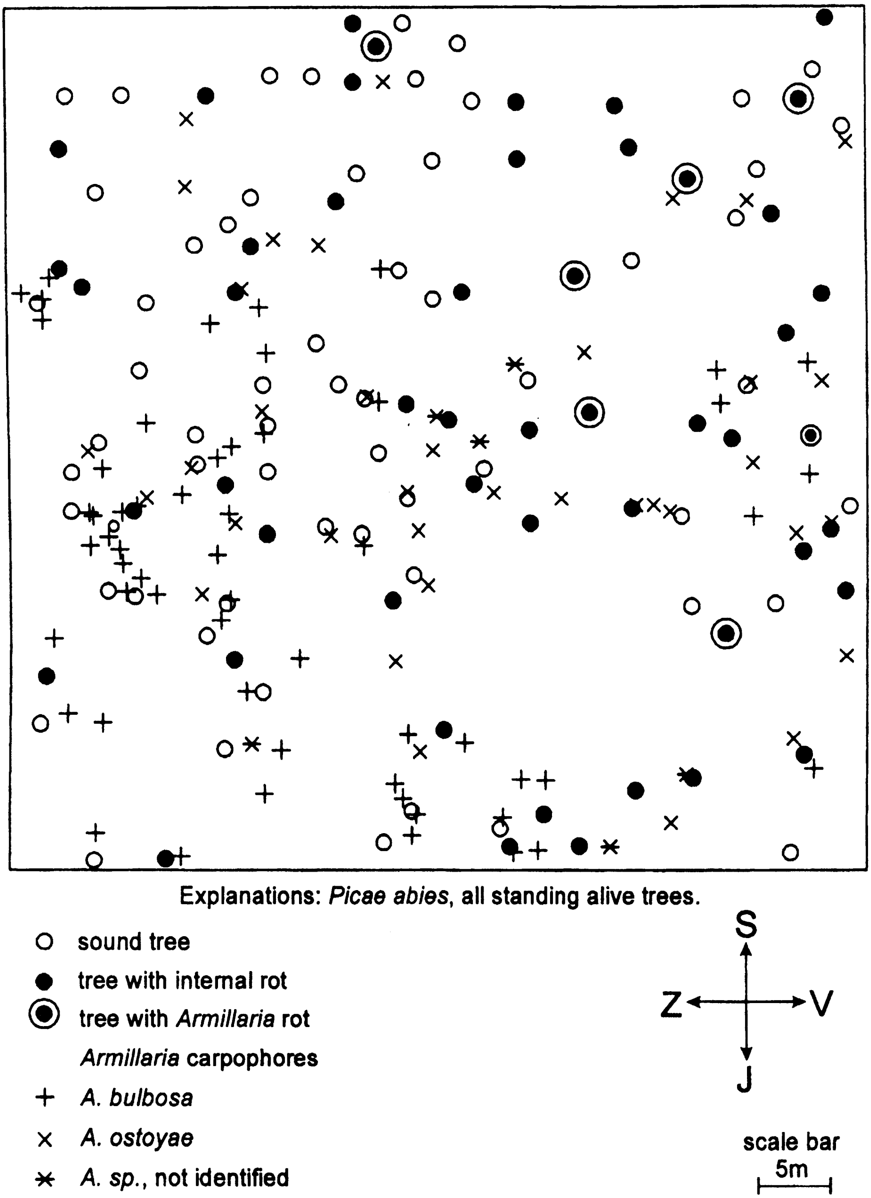

Fig. 1. – The distribution of trees and *Armillaria* carpophores on the Stožec permanent plot in 1993.

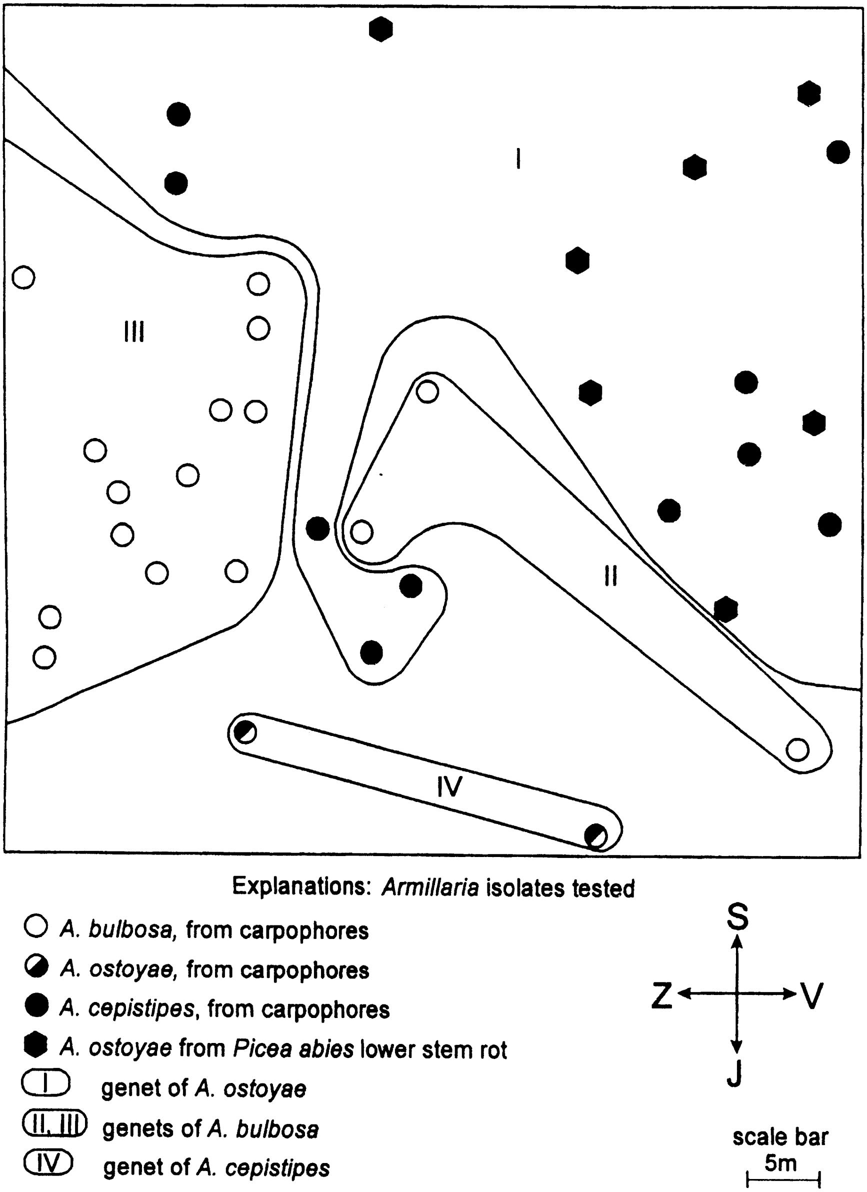

Fig. 2. – The distribution of *Armillaria* genets on the Stožec permanent plot in 1993.

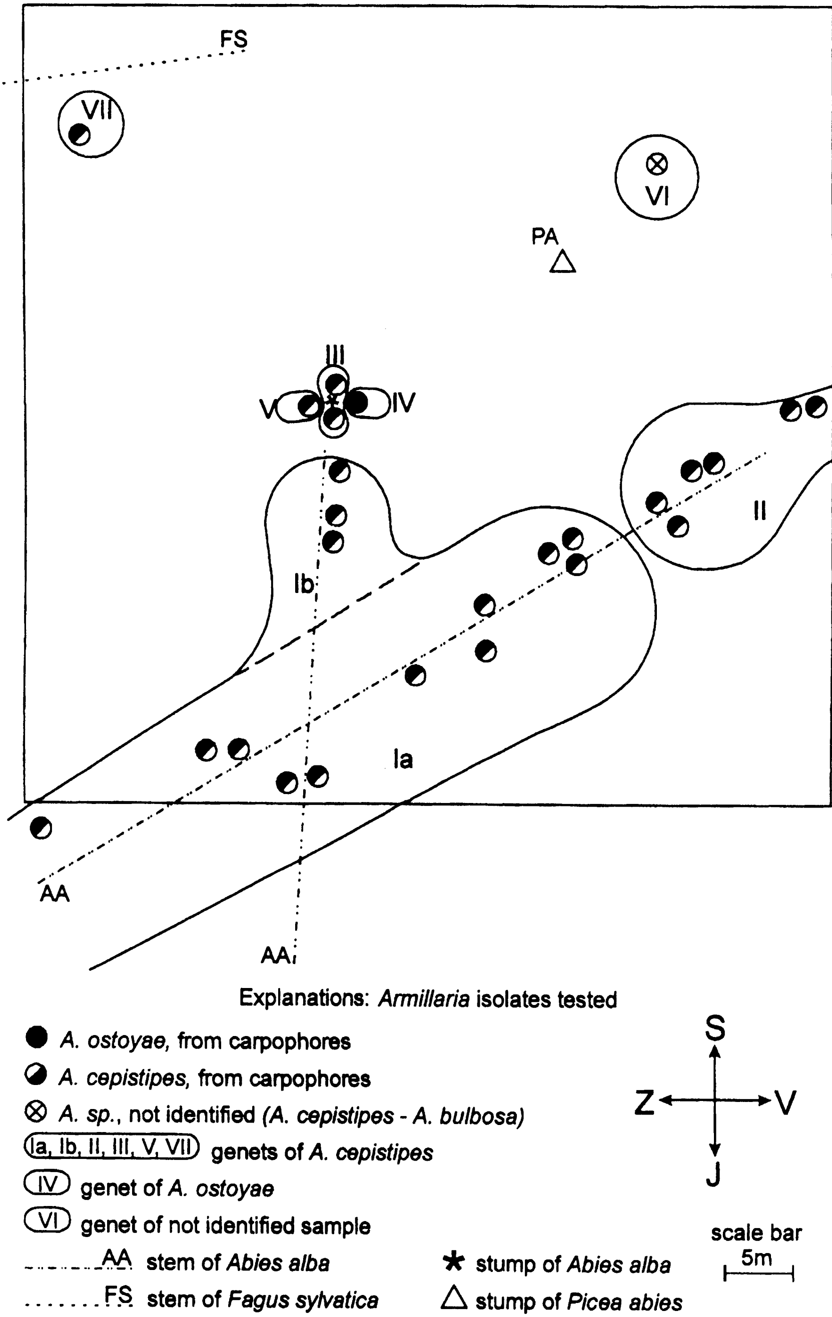

Fig. 3. – The distribution of *Armillaria* genets on the Žofínský prales **Id** permanent plot in 1994.