Preliminary studies on the relationship between *Tuber melanosporum* and vesicular arbuscular mycorrizhae in the "burnt-places"

ALEXIS ROSELL ARMENGOL

Resum

ROSELL ARMENGOL, A. (1997). Estudis preliminars sobre les relacions entre *Tuber melanosporum* i les micorrizes vessículo-arbusculars als tofoners. Collect. Bot. (Barcelona) 23: 41-46.

S'ha examinat l'estat de les micorrizes vessículo-arbusculars de les plantes herbàcies que viuen dins i fora dels tofoners deguts a *Tuber melanosporum* Vitt., a fi de determinar si el fong interfereix en la formació endomicorrízica. Quan varen comparar-se les plantes que sobrevivien als tofoners amb les del terreny del voltant, es va observar una clara reducció en llur creixement i en la micorrizació vessículo-arbuscular; i , a més, es va detectar una reducció notable en el nombre d'espores endogàmiques presents al sòl. Tot això suggereix que *T. melanosporum* produeix substàncies inhibents també respecte als fongs endomicorrízics.

Summary

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The vesicular-arbuscular mycorrhizal status of the herbaceous plants living inside and outside the "burnt- places" caused by *Tuber melanosporum* Vitt. was examined in order to determine whether the fungus may interfere with endomycorrhizal formation. When plants surviving in the burnt-out areas were compared to those in surrounding ground,a clear reduction in growth and vesicular-arbuscular mycorrhizal colonization were observed; additionally a reduction in the number of endogonaceous spores occurring in the soil was detected. These results suggest that *T. melanosporum* produces substances also inhibitive to endomycorrhizal fungi.

INTRODUCTION

The black truffle (*Tuber melanosporum* Vitt.) is an edible hypogeous fungus in much demand in southwest European countries for its gastronomic qualities. Its cultivation appears difficult and limited by its biological cycle (DELMAS,1978). It is ectomycorrhizal with

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various trees and shrubs including species of *Carpinus, Cistus, Corylus* and *Quercus,* (CERUTI, 1960; TRAPPE, 1962). Usually, when the mycelium of *T. melanosporum* develops in the soil and forms abundant mycorrhizae and sporocarps, almost all the herbaceous vegetation around the mycorrhizal plant disappears. This dimly understood phenomenon is known as the truffle-burn (CHATIN, 1892; DELMAS, 1978). However, some shrubs and herbs survive inside the burnt places, but they are stunted compared to the same species living outside these areas (MONTACCHINI & CARAMIELLO LOMAGNO, 1977). These resistent plants, such as *Briza maxima, Scleropoa rigida, Vicia tenuifolia,* are potentially vesicular-arbuscular mycorrhizal (VAM) (GERDEMANN,1968). The benefical effects of the VA endophyte on plant development are well known (TINKER, 1978; ALLEN, 1991). The objective of this study was to determine if the mycelium of *T. melanosporum* inhibits root infection by endomycorrhizal fungi.

MATERIAL AND METHODS

Hazel-nut trees were inoculated with *Tuber melanosporum* in the greenhouse under fairly controlled conditions and after three years transplanted to the field in a previous experiment made by DR. M. Palenzona (Istituto Nazionale per le Piante da Legno e l'Ambiente, Turin) An evident decrease of the herbaceous vegetation was observed around many of the trees, two years later, in 1979 . Then, two trees were selected for the study of mycorrhizal roots of herbaceous plants living inside and outside the truffle burns. The first tree produced a pound of sporocarps by yuletide and had a truffle burn within a radius of at least 1 m from the trunk. The second tree did not produce truffles and was surrounded by a feeble burn. The herbaceous cover ranged from 30% (first tree) to 50% (second tree) inside these burns, while all around the nonwooded surrounding areas was of 100% .

Three specimens of each sample were randomly collected, with associated soil, in June, August and October, inside, and 1 m outside the periphery of the burns. Many plant species occurred only outside the perimeter and therefore were excluded from the study.

In the laboratory, shoots of the plant specimens were measured and the roots were cleared and stained for assessment of VAM colonization (PHILIPS & HAYMAN, 1970).

The percentage of colonization of each plant was based on the presence or absence of the endophyte in 100 segments 1 mm long, randomly selected from the secondary roots, modifying the method of READ *et al.*, (1976). The degree of colonization was arbitrarily estimated as low, medium or high, depending on the number of endomycorrhizal hyphae cells in each segment. Arbuscules and vesicles were counted in each segment. Sporadic colonization by *Glomus tenuis* (Greenhall) Hall and other recognizable parasitic fungi were estimated separately (*Rhizoctonia, Olpidium, Phialophora*) .

Soil (B) samples, each from 1 to 10 cm deep,were collected inside and outside the first truffle burn and divided into five 50 g subsamples and examined for spore populations. Spores were extracted by wet sieving and decanting (GERDERMANN & NICOLSON,1963) through 350, 250 and 105 μ sieves. Aliquots of the lower 105 μ suspension were saved for counting the smallest spores.

Afterwards, a non statistical valuable experiment was made in climatic chamber with *Achillea millefolium* plants, mycorrhized or not with *G. mosseae* in controlled conditions. They were exposed, or not, to *T. melanosporum* exudates in order to confirm the field hypopthesis.

RESULTS

Nothing unexpected happened in the climatic chamber. Hosts without endophytes grew together fine. *Achillea* plants mycorrhized with *Glomus mosseae* grew also fine, but, afterwards, they stunted in contact with *Tuber melanosporum* exudates or *Quercus humilis* mycorrhized with this *Tuber*. However, non endomycorrized *Achillea* plants stunted much before when trying the same with them.

In the field, the herbaceous plants collected from **inside** the truffle burns were consistently smaller in height than those collected outside the burns, at all sampling times.

Plants from **outside** both truffle burns were typically colonized by VA fungi. However, in *Agrimonia eupatoria* and *Salvia pratensis* the intensity of colonization was greater than on the average, and in *Silene vulgaris* lower than the typical rate, and in *Brachypodium pinnatum* was inconsistent in the first area (very low on the spring and very high the rest of the year) and normal (70-98%) in the second tract.

The intercellular and intracellular endophytic structures of all the samples from **outside** the truffle burns were examined. Vesicles as well as arbuscules were very frequent. *Glomus tenuis* mycorrhizal colonization, easily recognized by its fine hyphae (HALL, 1977), was observed specially in *B. pinnatum* (15%) and other plant species.

Inside the truffle burns the roots of the plants displayed low VAM colonization, both in percentage and in intensity. Extraradical mycelia and intercellular hyphae were frequent, while arbuscules were rare. *G. tenuis* colonization was rarely observed. *Rhizoctonia* infections were extensive in all samples.

Spore Populations: Healthy appearing spores extracted from soil normally had yellow or light-brown walls, and contained abundant globules of lipids. Most spores were 60- 100 μ in diameter, and were identified as *Glomus fasciculatus* (Thaxter) Gerdemann & Trappe. However, some few light-yellow spores, 16-20 μ diameter, were tentatively identified as *Glomus tenuis*. Spore numbers averaged 44/g of dried soil outside this truffle burn, and 23/g inside.

These results obtained do support the working hypothesis that *T. melanosporum* initiates soil changes which favour nonmycorrhyzal plants, or low dependent ones, over VAM depending hosts. However, the result does not break down the chicken and egg situation and we may always wonder if the VAM colonization is scanty because the plants are feeble, or the other way round.

DISCUSSION

The reduction of the plants observed around hazel-nut trees and inoculated with black truffle, were similar to the truffle burns observed under natural conditions (CHATIN,1892). *Tuber melanosporum* seems to be the cause because, for example, *Corylus avellana* colonized by *Tuber albidum* do not show such vegetation changes.

Plants within the burn area are smaller and constantly have lower VAM root colonization, than those of the same species outside. Moreover, the roots of the plants living in the truffle burns had lower VAM colonization, fewer arbuscules - the primary sites for nutrients exchange between host and endophyte (COX & TINKER, 1976)-, and more abundant weak pathogens than the roots of the plants outside the burns.

Two species (*B. pinnatum* and *S. vulgaris*) display no statistically significant differences in VAM root colonization percentages between outside and inside the burns. The latter plant is considered a member of a nonmycorhizal family *(Caryophyllaceae),* and the former is according to our pattern in August and October, but not so clearly in June, when colonization seems just to begin. Maybe the outside sample was collected too near the periphery (see general numeric table). At some other few places *B. pinnatum* does not mark, exceptionally, the presence of a productive truffle burn area.

	A	B	C	D	E	F	G	H	I	J	K	L	M
FIRST TRUFFLE BURN													
Salvia pratensis I (outside)	71	29	35	12	24	17	13	71	44	39	67	0	0
(inside)	0	100	0	0	0	0	0	0	0	0	70	0	0
Salvia pratensis II (outside)	100	0	18	69	12	54	39	100	78	49	4	0	0
(inside)	0	100	0	0	0	0	0	0	0	0	92	0	0
Salvia pratensis III (outside)	97	3	41	46	10	13	10	97	83	3	12	0	4
(inside)	21	79	7	12	2	4	6	21	2	8	38	0	0
Brachypodium pinnatum I (outside)	5	95	3	2	0	3	2	5	1	0	12	0	1
(inside)	0	100	0	0	0	0	0	0	0	0	22	0	0
Brachypodium pinnatum II (outside)	78	22	43	28	7	5	19	77	55	1	12	1	0
(inside)	8	92	6	2	0	0	0	8	7	0	9	0	0
Brachypodium pinnatum III (outside)	92	8	57	31	4	4	3	87	76	0	4	0	1
(inside)	2	98	2	0	0	0	0	2	2	0	17	2	3
Agrimonia eupatoria II (outside)	96	4	62	34	0	6	0	96	81	18	12	0	0
(inside)	6	94	6	0	0	0	0	4	6	0	69	0	0
Silene vulgaris II (outside)	21	79	21	0	0	0	0	20	8	0	8	0	0
(inside)	0	100	0	0	0	0	0	0	0	0	20	0	0
Silene vulgaris III (outside)	44	56	44	0	0	15	15	44	44	0	0	0	0
(inside)	2	98	2	0	0	0	0	2	0	0	7	0	0
SECOND TRUFFLE BURN													
Arrhenatherum elatius I (outside)	94	6	46	40	8	57	16	92	50	31	8	0	0
(inside)	4	96	3	1	0	4	1	4	4	2	98	0	0
Arrhenatherum elatius II (outside)	87	13	73	11	3	20	9	87	70	8	6	0	0
(limit)	65	35	52	12	1	14	6	60	52	5	1	0	0
Arrhenatherum elatius III (outside)	56	44	33	18	5	5	5	53	32	33	4	0	0
(limit)	56	44	47	8	1	3	2	55	42	11	0	0	2
Achillea millefolium I (outside)	68	32	46	19	3	42	15	66	28	5	34	6	0
(inside)	15	85	15	0	0	1	3	15	6	0	6	16	0
Achillea millefolium II (outside)	64	36	45	15	4	20	19	64	32	6	6	24	0
(inside)	15	85	15	0	0	6	6	15	9	4	76	9	0
Achillea millefolium III (outside)	79	21	28	34	17	0	0	79	72	60	6	32	0
(inside)	2	98	2	0	0	0	0	2	2	0	56	0	0
Leucanthemum vulgare II (outside)	100	0	18	60	22	34	28	99	91	45	0	0	0
(inside)	16	84	10	6	0	4	4	16	16	2	76	0	0
Leucanthemum vulgare III (outside)	92	8	60	30	2	7	8	92	44	71	0	0	0
(inside)	26	74	14	8	4	11	12	26	16	14	56	0	0
Daucus carota II (outside)	98	2	80	18	0	48	39	98	54	5	0	0	0
(inside)	42	58	33	9	0	6	5	40	25	6	29	20	0

General numeric Table: Percentages among 100 analyzed segments. I: June 1979; II: August; III: October (sampling dates). **Legend**. A: Mycorrhized, B: Non mycorrhized, C: Scarcely mycorrhized, D: Moderately mycorrhized, E: Very mycorrhized, F: External mycelium, G: Penetration points, H: Internal mycelium, I: Vesicles, J: Arbuscules, K: *Rhizoctonia*, L: *Olpidium*, M: *Phialophora*

The results suggest that the reduced VAM root colonization may be caused by an antifungal activity of *T. melanosporum* (PAPA & PORRARO, 1978/79). This is also indicated by the reduced spore counts from the first truffle burn (the one wich produced truffle sporocarps) comparing it to the outside counts. The production of toxins by *T. melanosporum* has been proposed to explain the different microbial and fungal populations outside and inside truffle burns (CHALVIGNAC *et al.*, 1959; LUPPI MOSCA, 1972; LUPPI MOSCA & FONTANA, 1977). MONTACCHINI & CARAMIELLO LOMAGNO (1977) have suggested a direct activity against some herbs typically occurring on truffle producing zones, because aqueous extract of truffle sporocarp strongly inhibits seed germination.

CONCLUSION

Our results on the VAM status of the herbaceous plants agree with the concept that toxins produced by *T. melanosporum* act against the mycorhizal endophytes. Or at least there is a close interdependence among the below-grownd elements (ecto-mycorrhizal fungus, VA endophyte, weak pathogens) and hosts (endo-and ectomycorrhizal).

The degree of mycorrhizal dependence differs among hosts (GERDEMANN, 1975). Studies in naturally producing truffle zones (MONTACCHINI & CARAMIELLO LOMAGNO, 1977) established the presence of *S. vulgaris* inside the truffle burns, and *Cruciferae,* and other *Caryophyllaceae,* both typically nonmycorrhizal families acccording to GERDEMANN (1968). Moreover, typically VAM dependent species in the *Graminaceae, Leguminosae* and *Asteraceae* become stunted in the truffle burns.

Nevertheless, it might be questioned if the low levels of VAM colonization arise by toxic effects upon herbaceous plants, which would reduce assimilates supply to the roots, or, upon, directly, the VAM endophytes, and therefore originating as a result the weakness of the host.

In the field, previous conditions at the considered areas were homogeneous, except for the second area that was damper, but no main difference appeared between the two inside and outside zones.

According to Spanish truffle farmers the spreading of mineral manure onto the truffle burns may lead to the disappearance of truffle production. Therefore, future research would be carried out with very low quantities of mineral supply.

Direct confrontation between *T. melanosporum* and *G. mosseae* mycelia growing in vitro would be conclusive, but for the time being VAM fungi are hardly cultivable without plant host (SCHUBERT *et al.*, 1978).

ACKNOWLEDGMENT

To the C.S.M.T. (Centro di Studio sulla Micologia del Tereno), of the C.N.R. in Turin, and especially to Paola Bonfante Fasolo, who wrote the main part of the first manuscript.

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Acceptat: abril de 1994