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SOME OBSERVATIONS ON THE ESTABLISHMENT OF THE LICHEN *CALOPLACA AURANTIA* ON CONCRETE TILES IN ISRAEL

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Abstract. This paper investigates the colonization of concrete tiles by the lichen *Caloplaca aurantia* in a rural, non-polluted settlement in Israel. The percentage colonization by this crustose lichen on roof tiles 30, 45, and 60 years old was found to be 2.464 ± 0.732%, 22.972 ± 7.311% and 48.515±6.781% respectively. Scanning Electron Microscopy (SEM) revealed that spherical cells of a unicellular green alga, probably *Trebouxia* colonize pits on the weathered surface of the very same concrete tiles, as do also the ascopores of *C. aurantia.* In many of the pits, the ascospores arrange in clusters of 8 units. Fungal hyphae were observed close to ascopores and to the free algal cells.

Introduction

The roof tiles on houses built in Israel during the present century are most commonly made of concrete. Many such tiles, which are currently 60-70 years old, are covered mainly with the lichens *Caloplaca aurantia* (Pers.) var. *aurantia, Lecanora dispersa* (Pers.) Sommerf. f. *dissipata* (Nyl.) B. de Lesd., *Protoblastenia immersa* (Web.) Stein and *Candelariella aurella* (Hoffm.) Zahlbr.

The present study was a part of a wider investigation on lichen formation in nature. The focus here was on epilithic crustose lichens, making use also of Scanning Electron Microscopy (SEM). Another goal was to estimate the rate of coverage of concrete tiles by epilithic lichens in a rural non-polluted settlement in Israel.

Materials and methods

The observations relating to the present investigation were made in a rural settlement of about 8000 inhabitants (Magdiel, Hod Hasharon) located 20 km. NE of Tel-Aviv. Tile coverage by *Caloplaca aurantia* was assessed by photographing a 50 x 50 cm area on the west-facing slope of selected roofs of one-storey buildings. To do this in each case, we used a wooden frame (50 x 50 cm.) which was temporarily affixed to the roof surface. Color photographs of the areas enclosed by such wooden frames were obtained and these were then copied by a document

photocopy machine, enlarged two-fold, and weighted precisely by an electronic balance. Any areas of the photocopies showing thalli of *C. aurantia* were carefully snipped out with scissors and weighted separately. The percentage of the lichen coverage was then calculated by the formula:

$$\frac{\textit{Weight of the paper representing the lichen area}}{\textit{Weight of the paper representing the entire 50x50 cm. area.}} \times 100$$

The roofs selected for photography were 20, 45, and 60 years old. Ten roofs from each of these age categories were sampled by three different photographs per roof, yielding a total of 90 photocopies.

In order to ascertain whether free components of lichens existed on the photographed roof tiles, we actually collected samples of tiles from the west-facing slopes of Magdiel's 30,45 and 60 year old roofs.

In all these tile samples, the lichen coverage was less than complete, which enabled easy study of bare areas on which lichenization could occur.

The collected tiles were next fractured to yield bare chunks of about 0.5x0.5 cm each, the idea being that free lichen components and contacts between ascospores and photobiont cells would be detectable close to the established thalli of the nearest lichen colonizing the tile shards.

Samples from these seemingly bare fragments of tile were fixed overnight with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, washed with the same buffer, dehydrated by increasing concentrations of ethanol, dried with a critical point drier and finally coated with gold. The samples were then subjected to a Jeol-35 scanning electron microscope operating at 25 KV.

Results

From a chronological standpoint, the rate of lichen colonization on the tiles during the first 30 years was found to be relatively slow (2.464 ± 0.732%) but this increased to 22.972 ± 7.311% after 45 years and attained 48.515 ± 6.781% after 60 years. The observed differences in the coverage percentage were highly significant by Student's t-test for all the three age-categories of tiles ($p < 0.001$).

Fig. 1 - Fractured apothecium of *Caloplaca aurantia* growing on a 30-year-old concrete tile. Bar = 100 μm.

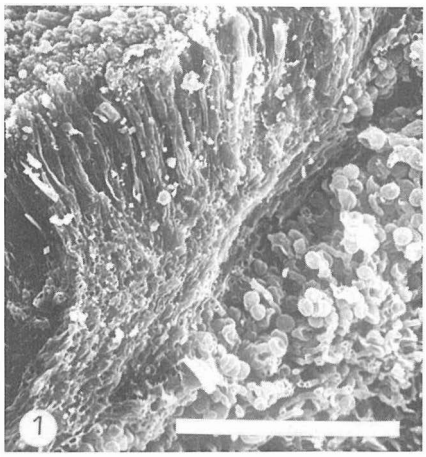
Fig. 2 - Photobiont and mycobiont cells as in Fig. 1 but shown in higher magnification. Bar = 10 μm.

Fig. 3 - Ascus and paraphyses of the hymenium of *C. aurantia*. Bar = 10 μm.

Fig. 4 - Immature *C. aurantia* ascospores still in the ascus. Bar = 10 μm.

Fig. 5 - Upper surface of a seemingly bare part of a 30-year-old concrete roof tile. Note the rough outer surface of the algal cells. Bar = 10 μm.

Fig. 6 - A group of unicellular algal cells in a pit on the surface of a 45-year-old concrete tile. Bar = 10 μm.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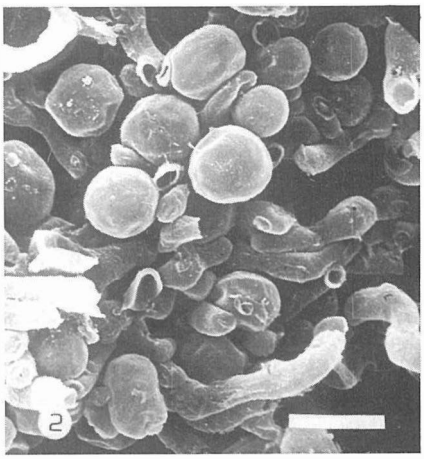
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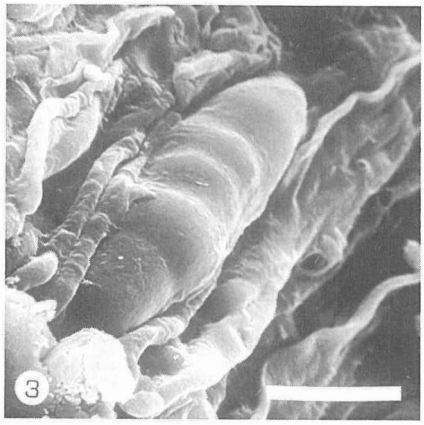
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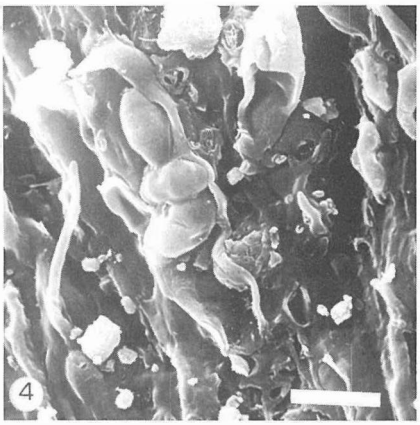
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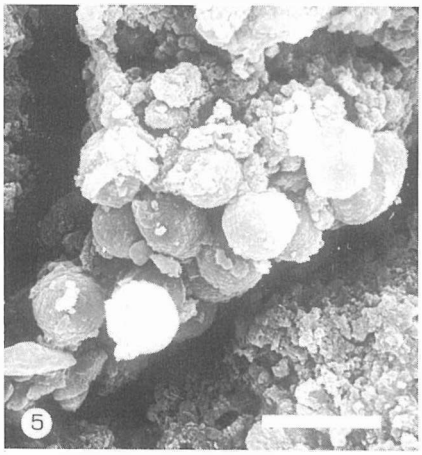
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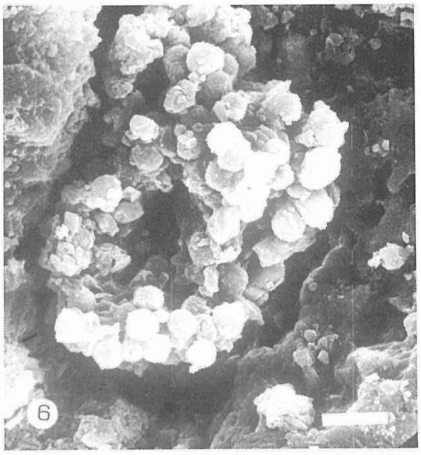
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The coefficient of variation, C.O.V., $\frac{(S.D.)}{\bar{x}}$ was 0.29 for the 30 years-old tile, 0.31 for the 45 year-old tile and 0.13 for the 60 year-old tile.

Fig. 1 offers a SEM view of a fractured apothecium of the lichen *Caloplaca aurantia* growing on a 30 year-old concrete tile from a roof in Magdiel. This figure shows the spherical cells of the unicellular green alga *Trebouxia* as well as a lateral view of the hymenium and a part of the disc surface. The sizes of the algal cells are presented in a higher magnification in Fig. 2, where they are seen to vary between 5 and 10 μm. One ascus and several paraphyses are shown in Fig. 3, while three ascospores, probably immature, are shown in Fig. 4.

A view of the bare surface of a concrete tile picked from among established colonies of *C. aurantia* is given in Fig. 5. A spherically-shaped unicellular green alga was found to colonize pits on the weathered surface of such tiles. These algal cells, the only kind of free unicellular green alga on the tiles, measured 5.5 - 8.5 μm in diameter and invariably displayed a rough external surface (Figs. 5-10, 13-15). As can be noted from Fig. 5 and 6, tile surfaces are smooth on their inside, probably due to the abrading chemical action of the algal lithobionts on the carbonates of the concrete tile.

The established colonies of *Caloplaca aurantia* bear numerous apothecia. These produce large numbers of ascospores which are entrapped in pits on the surface of the 60 year-old tiles (Fig. 7) as well as on those aged 30 and 45 years (Fig. 8 and 9 respectively). The spore shapes and sizes match those given by Galun (1970) for the ascospores of *Caloplaca aurantia.* In many of the pits on the bare upper surface of tiles taken from 30, 45 and 60 year-old roofs, clusters of 8 ascospores can be seen (Figs. 10-12). Frequently fungal hyphae (or ascospores) and free algal cells are seen in the same pit (Figs. 12-14). Some ascospores with shrunken walls

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Fig. 7 - Free algal cells, presumably of *Trebouxia*, and an ascospore of *C. aurantia* inside a pit of a 60-year-old concrete tile. Bar = μm.

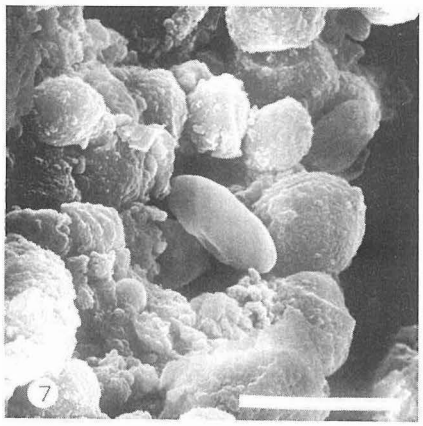
Fig. 8 - An ascospore of *C. aurantia* and some algal cells inside a pit of another 30-years-old tile. Bar = 10μm.

Fig. 9 - Free photobiont and *C. aurantia* ascospore inside a large pit on the surface of a 45-year-old tile. Bar = 10 μm.

Fig. 10 - A view of a bare area on the upper surface of a 30-year-old concrete tile. Note 8 ascospores produced and dispersed by *C. aurantia* lying close to a group of algal cells growing in the same pit. Bar = 10 μm.

Fig. 11 - Eight ascospores of *C. aurantia* inside a large pit on a 45-year-old tile. Dust particles in a variety of shapes and sizes are deposited in the same microniche. Bar = 10 μm.

Fig. 12 - Eight ascospores of *C. aurantia* inside a large pit on a 60-year-old tile. Most of the ascospores are located in small depressions in the described niche. Note fungal hyphae in the same pit. Bar = 10 μm.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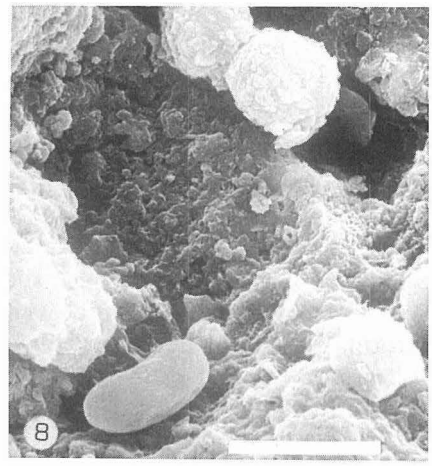
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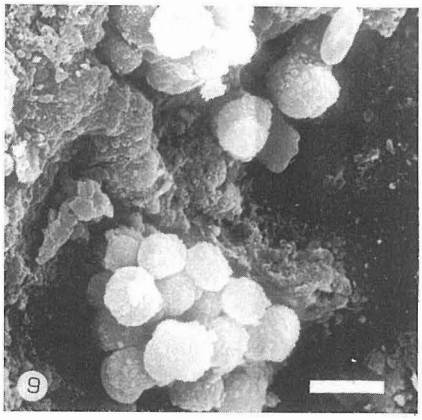
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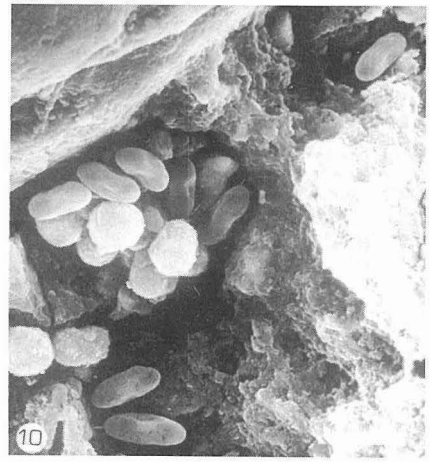
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can be observed as well (Fig. 15). Clusters of free *Caloplaca aurantia* ascospores are located, however, also in pits which are not colonized by algal cells (Fig. 16).

Discussion

It is especially noteworthy that the lichen coverage of the 30 year-old tiles was a mere 2.464 ± 0.732% whereas tiles which were 45 years old showed nine times as much coverage. This means that the dispersal of lichen components, i.e. of ascospores and algae, increases considerably during the 15-year span between the tile ages of 30 and 45. It is possible that the observed increase of coverage is associated with an enhancement of weathering processes on the tile surface, for pits and ruts are known to act as traps for dust, bird droppings, water, algal cells and ascospores.

In a roof-slope situation, this is especially important, because once the *C. aurantia* ascospores have dispersed in the pits they can germinate under favorable conditions.

The coverage of the 60 year-old tiles by *C. aurantia* is about twice as much that on the 45 year-old tiles. Possibly the slower lichen colonization rate on the tiles is linked to the morphology of the substrate surfaces, for we have noted that on old tiles (50-60 years old) the pits and ruts are larger and bigger than on younger tiles. We have indeed never observed a lichen coverage of 100% on any of the 70 to 75 year-old tiles in the studied rural and non-polluted area. Many of the roofs here were in fact hardly colonized by lichens.

All this suggests that because of the enhanced weathering processes, the pits and ruts on very old tiles become too big, so that lichen-free components can no longer be trapped on the roof slopes, especially during heavy showers.

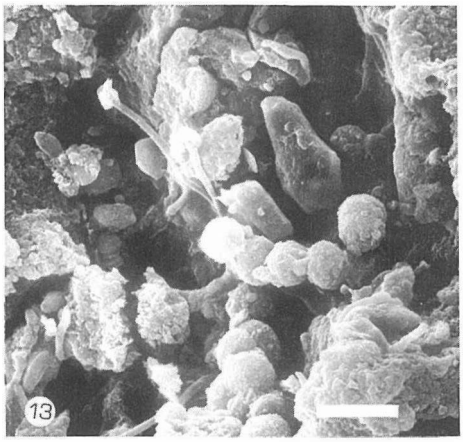
The fact that lichen ascospores were often found in clusters of eight or thereabout agrees very well with the findings of Bailey and Garrett (1968) that under laboratory conditions the usual number of *C. aurantia* ascopores per projectile is eight. Evidently, under natural conditions as well, these ascospores are liberated from the asci of *C. aurantia* in clusters of eight. We further believe that the free-living algal cells with the rough surface which we encountered were those of *Trebouxia*. Indeed, free colonies of *Trebouxia* have been reported by Nakano (1971 a; 1971 b), Tschermak-Woess (1978) and Bubrick et al. (1984).

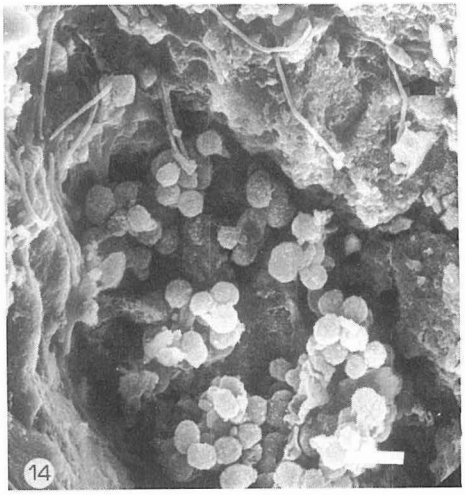
Fig. 13 - Algal cells, fungal hyphae and dust particles inside a large pit on a 60-year-old tile. Bar = 10 μm.

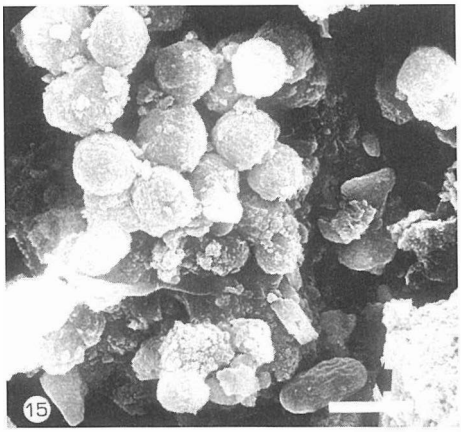
Fig. 14 - *C. aurantia* ascospores (arrow), hyphae and algal cells inside a pit on a 45-year-old tile. Bar = 10 μm-

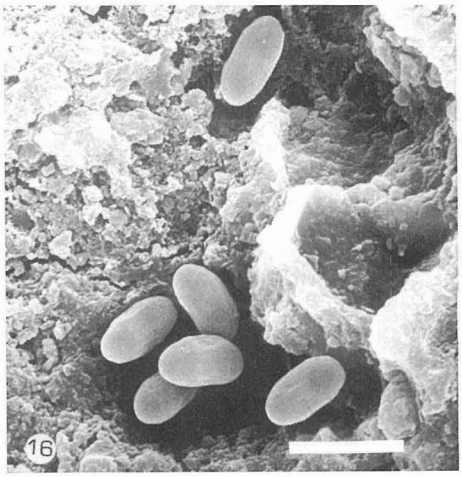
Fig. 15 - Algal cells (note their rough cell surface) and one ascospore of *C. aurantia* with shrunken walls. Bar = 10 μm. The substrate is 45 years old.

Fig. 16 - A cluster of 6 ascospores of *C. aurantia* located in a pit which is not colonized by algal cells. The substrate is 60 years old. Bar = 10 μm.









Our present findings raise two interesting questions, namely:

1) Does the rough outer surfaces possessed by the free-growing algae in the present study play a role in the recognition between these cells and the cohabitant fungal hyphae produced by the germinating ascospores of *C. aurantia*?

2) Is the outer wall surface of these algae rough because it contains binding compounds that are related to mycobiont-phycobiont recognition?

In reviewing the relevant literature, we find that Bubrick and Galun (1980 a) observed, by cytochemical means, two wall layers in trebouxioid phycobionts, and the same was observed also via cytological methods by Honneger (1982). The latter author detected protein-like particles embedded in an amorphous matrix in the outer wall of cultured *Trebouxia* cells (Honneger 1982). Subsequently she ob-

served a similar amorphous matrix also in the large mature cells of the trebouxioid phycobiont in *Cetrelia olivetorum* prior to autospore formation (Honneger, 1984. Fig. 4A).

Finally, a SEM micrograph of a group of developing aplanospores of a *Trebouxia* in the *Hypogymnia physodes* thallus, reveals that the surfaces of these cells have a rough outer coating (Fiechter and Honneger, 1988).

It is tempting therefore to conjecture that the rough surface displayed by the free unicellular green algae in the present study indicates that these cells are *Trebouxia* cells originating from established thalli of *C. aurantia.* According to Ahmadjian (1980) free-living photobionts indeed could be microcolonies derived from woospores of algae within existing lichenized associations. Hawksworth and Hill (1984) maintain that photobiont cells may be acquired:

a) from free-living algae
b) from existing thalli of other lichens or
c) from the vegetative propagules of other lichens.

The structure and composition of the cell wall surface differs in *Trebouxia* phycobionts when in symbiosis than when in the cultured, non-symbiotic state (Honneger, 1984). Similarly, differences in the cell wall surface composition between cultured and symbiotic *Trebouxia* phycobionts of *Xanthoria parietina* have been demonstrated by Bubrick and Galun (1980 b) and by Bubrick et al. (1982) via histochemical and immunological methods.

Previously, we have shown (Garty and Delarea, 1987) that on concrete tiles collected at another rural site (Ganne Am, Israel), the *Caloplaca aurantia* ascospores germinated in proximity to free algal cells very similar to those described in the present study.

Our present observations on the algal wall surface raise, however, further questions: Why is the outer surface of the *Trebouxia* cells shown in the apothecial section (Figs. 1 and 2) smooth? Is this smoothness related to fact that in this part of the lichen the fungal hyphae are relatively few? Could we conclude that these photobiont cells have very little to do with recognition of fungal hyphae? Be that as it may, we note that in the present study the algal cells of *Trebouxia,* as shown in Figs. 1 and 2, appear different both from our own free algal cells as well as from the *Trebouxia* and trebouxioid cells of other investigations cited by us, which act as photobionts in the vegetative thalli.

Several of our micrographs show numerous fungal hyphae inside the pits and ruts on the tile surfaces. In epiphytic lichens it has been shown (Jahns et al., 1979) that the algal cells form clumps that are intimately associated with long fungal hyphae, the latter probably deriving from spores of the lichen *Lecanora varia,* dispersed on needles of the tree *Picea abies.* Germinated spores of the epiphytic foliose lichen *Xanthoria parietina* have been observed on tree bark and, in some cases, in close proximity to *Trebouxia* and/or *Pseudotrebouxia* cells (Bubrick et al., 1984). We believe, therefore, that on progressively older tiles the presence of fungal hyphae in the same microniches as algal cells probably signifies the initiation of lichenization.

Acknowledgement

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