The Use of Mycorrhizal Fungi During Propagation

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INTRODUCTION

It will be well known to some nurserymen that many of the Cupressaceae family of plants are somewhat slow and difficult to propagate (Blythe, 1989). Callus tissue readily forms on the base of cuttings, gradually turning from a white to dark-brown colour, growing slowly larger to a final size of up to 20 mm in width. The cutting may remain in this state for over 12 months, the foliage still retaining a healthy appearance. This problem is also common in other genera, such as, *Grevillea* and *Hakea*. Our interest in this phenomenon arose after the discovery of a seedling variant of *Cupressus arizonica* var. *glabra* named 'Limelight' in the nursery 9 years ago. It also has a tendency to form a callus without readily forming roots. This paper reports the use of mycorrhizal fungi as an aid to root initiation in *Cupressus arizonica* var. *glabra* 'Limelight' at both fresh-cutting and callused stages. In this paper we refer to two main types of mycorrhizae. These are ectomycorrhizae and endomycorrhizae—hereafter referred to as "EctoM" and "EndoM". The EndoM fungi penetrate roots to form characteristic intracellular bodies called vesicles and arbuscles, hence the term VAM applies to EndoM. Moisture and nutrients are transferred from the fungus to the plant. The plant roots provide a source of carbohydrate for the fungus. This is a beneficial (symbiotic) relationship which occurs in about 80% of all vascular plants (Malloch et al., 1980). The EctoM fungi do not penetrate living cells in the roots but instead surround them, forming a sheath which is visible to the naked eye. Moisture and nutrients are transferred as in EndoM. EctoM have a distinctive fruiting body similar to a mushroom, which protrudes above the soil-line. EndoM, however, are rarely visible to the naked eye.

MATERIALS AND METHODS

In 1992, when reading through the index of past I.P.P.S. Proceedings, I noticed that there were a few papers written on the use of mycorrhizal fungi in plant propagation (Linderman and Call, 1977; Dangerfield, 1975; Verkade, 1986). It was then decided to research the subject more thoroughly. Initial inquiries were made with Kevin Handreck at the I.P.P.S. Albury Conference in 1993 in an attempt to locate a source of mycorrhizal fungi. Dr. Clem Kuek, Senior Lecturer at the University of Western Sydney, was recommended. Dr. Kuek is well known for his work on EctoM fungi and the resultant use of the inoculum Mycobead in *Eucalyptus* plantations in Western Australia.

It was also recommended that we contact a company in the United Kingdom named MicroBio Ltd., who produce the EndoM inoculum Vaminoc. Through their extensive grower trial program throughout Western Europe, they demonstrated the benefits of using VAM at the propagation stage in coniferous plants (Cargeeg, 1994). Benefits included increased root and shoot dry weights and a reduction of certain pathogenic fungi. Although literature seemed to indicate that the genus *Cupressus* was host to the EctoM (Malloch et al., 1980), a research scientist for MicroBio, Mr. Piran Cargeeg, suggested we trial Vaminoc, which had proven

beneficial in the propagation of conifers in European studies.

Coinciding with this was the discovery of four naturally occurring EctoM fungi fruiting bodies (Basidia) around the roots of containerized 'Limelight' stock plants at Coachwood Nursery. These unidentified fungi were collected, numbered M1, M2, M3, and M4, and sent the same day to Dr. Clem Kuek who was able to culture each one on sterile agar plates for future inoculation. In all, five separate experiments were carried out during which it was decided to discontinue the use of M2 due to its inefficacy. This paper concerns one of these five trials.

In September 1994, an experiment was formulated to evaluate the effect of M1, M3, M4, and Vaminoc on the rooting of 'Limelight' cuttings. Both fresh and callused cuttings were tested. The presence of rooting hormone IBA 0.8% powder formulation was also tested to see whether or not this would enhance the effect of the mycorrhizal fungi on the cuttings. A bottom heat of 26C was maintained throughout in a plastic-covered propagation house with 50% shading. The cutting medium consisted of perlite, milled coconut fibre, and fine kaolite (6:2:2, by volume). One hundred cuttings were stuck in each tray and placed onto sand beds under intermittent mist. Fresh cuttings were collected the same day from hard-pruned, in-ground stock plants. Callused cuttings were collected the same day from trays of cuttings which had been stuck 10 weeks previously. After 10 weeks the cuttings were removed, washed, and inspected for the presence of roots. Those with roots were root pruned to within 1 cm of the stem and the roots weighed. Standard nursery hygiene was maintained at all times with no fungicides of any type being used to minimise any losses to the inocula.

PREPARATION AND INOCULATION OF MYCORRHIZAE

Ectomycorrhizal Fungi M1, M3, and M4. These three mushroom-type fungi (Basidiomycetes) were cultured on agar plates. Ten plates of each fungus were

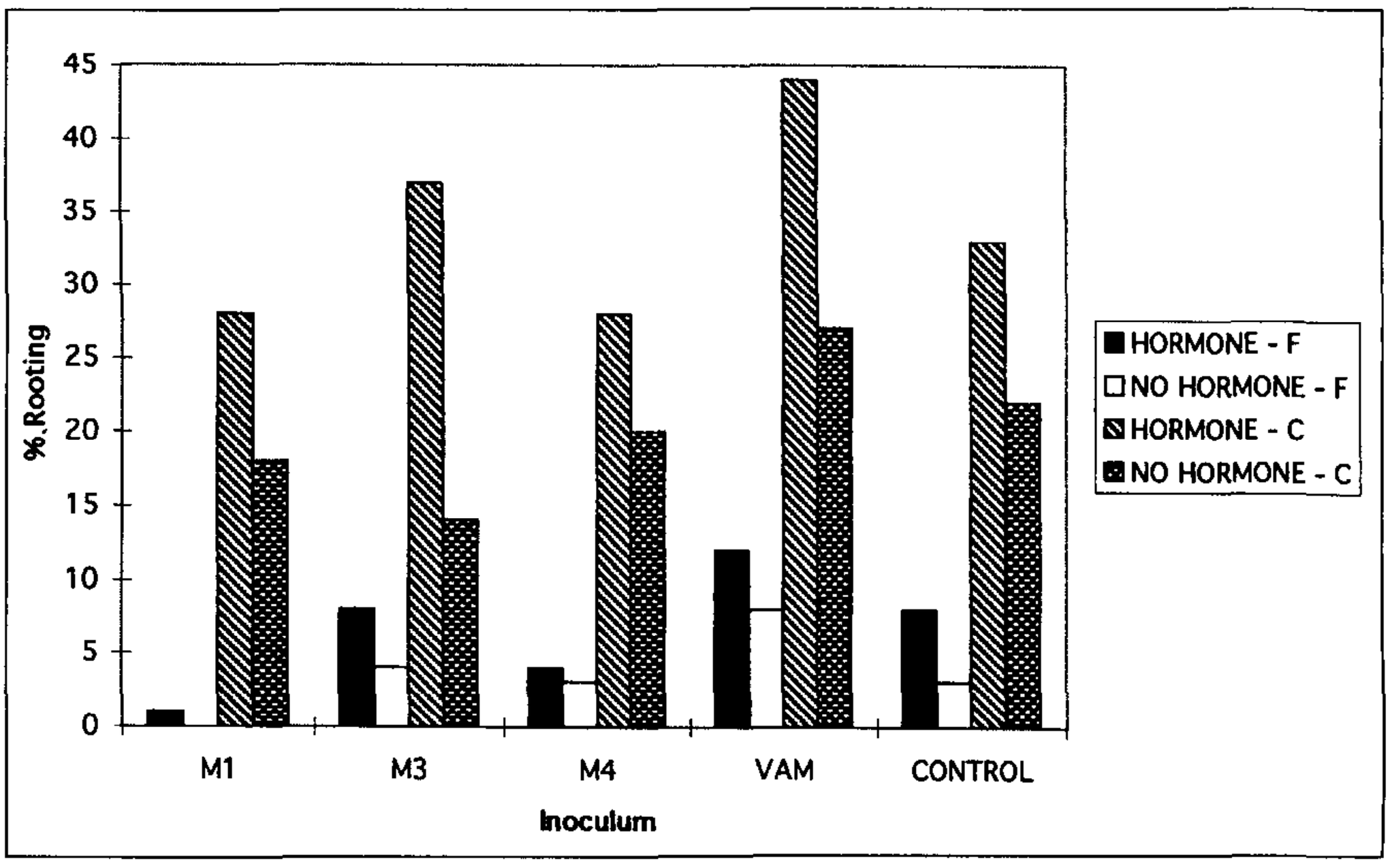

Figure 1. Effect of inocula on root formation (% rooting) with fresh (F) and callused (C) cuttings.

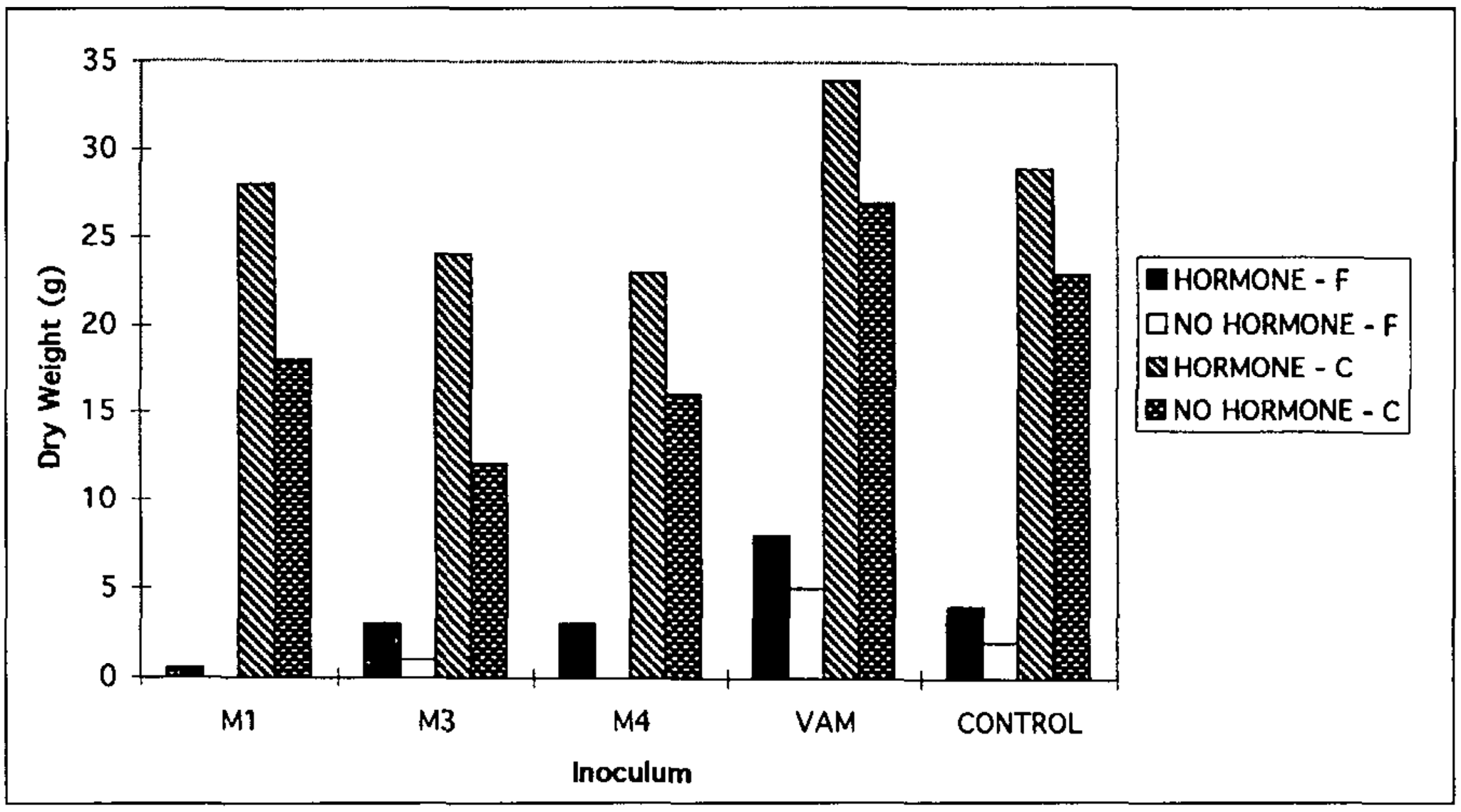

Figure 2. Effect of inocula on root formation (dry weight) with fresh (F) and callused (C) cuttings.

blended with 500 ml of rain water to produce a gel which could be poured directly into trenches. Cuttings were placed directly onto the gel and back-filled with cutting medium.

Endomycorrhizal Fungus Vaminoc. This commercial formulation was in granular form, 2 mm or less in size. It was advised in the guidelines sent to us to direct drill the granules into the cutting medium so that direct contact with the cuttings would be made. This procedure was followed by pouring Vaminoc into trenches, sticking the cuttings directly onto the granules, and back-filling. The recommended rate of 1 g per cutting was used.

RESULTS

The results of this experiment are shown in Figures 1 and 2. The addition of M1, M3, and M4 into the cutting medium did not result in an increase in rooting percentage or dry weight of roots compared to the control, except when M3 was used in combination with hormone on callused cuttings. In each case the addition of hormone increased rooting as expected, however, the cuttings with added M3 exhibited the greatest response. M1 and M4 were less effective than the control on fresh and callused cuttings, with or without hormone. On callused cuttings M4 was comparable to M1 in strike rate but M4 produced slightly less root dry weight. Vaminoc (VAM) was the only inoculum to give an increase in rooting percentage and dry weight compared to the control. VAM with hormone resulted in 12% rooting on fresh cuttings and 44% on callused cuttings. The control yielded 8% on fresh cuttings and 33% on callused cuttings. It was also observed that losses due to fungal infection decreased when the inoculum was present. VAM had almost no losses, M3 and M4 only some, while M1 was similar to the control.

DISCUSSION

The results show that rooting of 'Limelight' is greatest when previously callused cuttings are used. This is to be expected as callus formation is simply a stage closer

to root initiation than fresh cuttings. The fact that M1 and M4 suppressed root formation is interesting. It indicates that they may not form a mycorrhizal association with 'Limelight'. The fact that the fungi were growing in the containers of the plants may be due to the presence of composted pine bark and sedge peat in the potting media. This could provide the fungus with a short-term niche until a suitable host is found (Gianinazzi et al., 1986; Patterson et al., 1986). It would be difficult to explain the presence of the fungi in the containers any other way. They certainly could not have originated on the roots of the plants, as the plants had been grown in every stage in soilless media. The very fact that EctoM can be cultured *in vitro* indicates that they can exist for some time without a suitable host. Even if M1 and M4 are mycorrhizal with 'Limelight', they may be host-specific when it comes to root enhancement. This has been shown in previous trials (Linderman and Call, 1977).

The observed enhancement of rooting in M3 when hormone was added could be due to the catalytic effect which was observed by Linderman (1978). Mycorrhizae produce auxins, cytokinins, gibberellins, and B vitamins in vitro. The presence of one or more of these may in turn enhance mycorrhizal development. However, it is difficult to draw any firm conclusions in this area as the precise way in which mycorrhizae and growth regulators interact is still largely unknown. The fact that M1 and M4 showed a significant increase in rooting (much more pronounced than either M3, VAM, or control) when callused cuttings were used, indicates that these fungi need root tissue to become effective. Callus growth while not root tissue as such, is the stage immediately preceding it. Perhaps the fungi were able to extract the necessary nutrient from the callus to commence the symbiotic process which resulted in root formation. Careful examination under magnification may reveal this in future work.

The most successful inoculum in every case was Vaminoc. This result was not expected as previous literature had indicated that the genus *Cupressus* had a host-specific grouping of EctoM fungi (Malloch et al., 1980). However these results seem to concur with more recent work which suggests that some genera normally considered ectomycorrhizal are readily infected by VAM fungi, especially early in the growth phase (Cargeeg, 1994).The increase in percent rooting and dry weight occurred whether or not hormonewas used. It is possible therefore, that the VAM fungi produce root-promoting substances as mentioned earlier, or they infect the cells of the cutting/callus before roots form, resulting in increased rooting.

CONCLUSION

The use of M1, M3, and M4 in the form of agar gel is cumbersome and time consuming, and the results of the trials do not justify their use in 'Limelight' propagation. A more useable formulation such as Mycobead developed by Biosynthetica, would be necessary before commercial use could be considered. Further research needs to be carried out in relation to EctoM and its effect on root growth, particularly after root initiation, as this may be where its real value lies. Vaminoc in the granular form was easy to apply and gave an increase in rooting which justifies further investigation as an aid to propagating species known to be slow or difficult to propagate by conventional methods. The results of this trial indicate that there is significant potential in the ongoing use of mycorrhizal fungi in the field of plant propagation and in the horticultural industry generally.

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