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Effect of ultraviolet disinfection of hydroponic solutions on *Pythium* root rot and non-target bacteria

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Abstract

Dispersal of root pathogens is a major concern in closed hydroponic cultures. To limit dispersal, ultraviolet (UV) disinfection technology has been used to remove pathogens but its effect on non-target bacterial populations is largely unknown. In this study, the circulating solution was disinfected with various UV doses (i.e., 19, 38, 59, 88 mJ cm^{-2}) before recirculation. At the beginning of the experiment, the hydroponic solution was inoculated with *Pythium aphanidermatum* at 6.7 ± 1.5 CFU mL^{-1}. Four months later the population density of *P. aphanidermatum* reached 1030 CFU mL^{-1} in the control and 1028, 970, 610, and 521 CFU mL^{-1} in the solutions treated by the above UV doses. In all UV-treated solutions, significant ($P < 0.05$) reduction of both *Pythium* and bacterial populations was noted. For the former, its reduction did not result in decrease of *Pythium* root rot nor increase of tomato fruit yield. For the latter, its reduction showed a concomitant decrease of the populations in the rhizosphere. The bacterial populations in the rhizosphere were 6.77×10^9 CFU mg^{-1} fresh roots at the beginning of the experiment and were 7.89×10^8, 9.93×10^7, 7.33×10^7, and 3.51×10^7 CFU mg^{-1} fresh roots at the end of the experiment in the control, UV38, UV59, and UV88 treatments, respectively. The bacterial density also decreased with time in the control (UV0) although at a low rate. The results suggest that the attempt to control *Pythium* root rot by UV irradiation of recirculating solutions to remove *P. aphanidermatum* also affects the non-target bacterial populations in the rhizosphere. The interaction between the target pathogen and non-target bacterial flora in UV-treated hydroponics needs further investigation.

Introduction

Dispersal of root pathogens is a major concern in closed hydroponics. Paludan (1985) described rapid spread of viral diseases via recirculating solutions. *P. aphanidermatum* (Edson) Fitzp. has been reported to cause root rots of tomatoes, cucumbers, and spinach in hydroponics (Jenkins and Averre, 1983; Stanghellini et al., 1984). It can be introduced into hydroponic cultures by infected seedlings, contaminated rockwool cubes, or infected water sources (Jarvis, 1996). Zoospores of *P. aphanidermatum* can be disseminated throughout the system with recirculating solutions (Stanghellini and Rasmussen, 1994). In order to minimize the dispersal of root pathogens there is a need to disinfect recirculating solution. UV irradiation has been tested for this purpose since the 1980s (Ewart and Chrimes, 1980; Menzies and Bélanger, 1996). Recently, Runia (1994) reported that UV at 100 mJ cm^{-2} reduce 99.9% of *Fusarium* spp., and 90% of tomato mosaic virus. For complete disinfection, however, a dose of 250 mJ cm^{-2} was needed (Runia, 1995).

UV irradiation reduced the populations of the target pathogen as well as the non-target bacteria in hydroponics. The bacterial density in recirculating solutions

is usually 10^5–10^6 colony forming-unit (CFU) mL^{-1} in a tomato hydroponics (Berkelmann et al., 1994). The number of bacteria on young tomato roots can be as high as 10^{10} CFU mg^{-1} fresh roots (Waechter-Kristensen et al., 1994). In a UV-treated hydroponics, however, bacterial density is reduced from 6.5×10^5 to 2.5×10^4 CFU mL^{-1} at 44 mJ cm^{-2} (Buyanovsky et al., 1981). The decline of bacterial populations in recirculating solutions may eventually affect their counterparts in the rhizosphere. Potential problems caused by such decline in UV-treated hydroponics largely remain unknown. The objective of this study is to determine the effect of UV irradiation on *Pythium* populations in recirculating solutions and on non-target bacteria in both recirculating solutions and the rhizosphere in a tomato hydroponic system.

Materials and methods

Experimental design and layout

Four UV lamps (Aqua UV712, 708, 705, 702, Trojan Technologies Inc., London, Canada) were assembled in a common sequential feeding line (Figure 1). UV irradiation was applied immediately after planting and continued until the end of the experiment. The feeding solution was passed through a membrane filter with a pore size of 100 μM. The flow rates of the recirculating solutions through UV712, UV708, UV702, and UV705 were 36, 27, 18, 9 L min^{-1}, respectively. The transmittance of the solutions was maintained above 50% at a wavelength of 253.7 nM throughout the experimental period. The irradiation dose from each lamp is a function of light intensity, transmittance, and the flow rate of the circulating solution. The four UV units each provided a dose of 18.9, 19.3, 21.1, and 28.6 mJ cm^{-2} for a single pass, respectively. The UV dose received by the feeding flux was a sum of the preceding units. Therefore, the feeding flux diverted into each block (i.e., block 1, 2, 3, 4, and 5) received an accumulated dose of 18.9, 38.2, 59.3, and 87.9 mJ cm^{-2}, consecutively, which were henceforth referred to UV19, UV38, UV59, and UV88. Solution diverted from entering the UV units was referred to UV0, in which the plants grown taken as the control. All treatments were arranged in a randomized complete block design and replicated in four rows, six plants per row. The solutions used for the plants grown in the guard rows were not infected with *P. aphanidermatum* and circulated separately. UV lamps and filter were cleaned at weekly interval. The transmittance of recirculating solutions was determined at a wavelength of 253.7 nM by a spectrophotometer (SP8-100 UV/VIS, Pye Unicam Ltd., Cambridge, England).

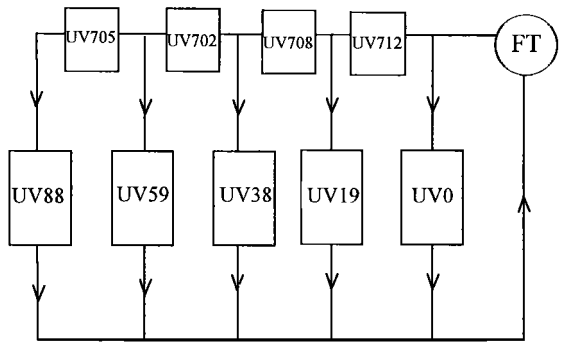

Figure 1. Diagram of the recirculating hydroponic system installed with multiple ultraviolet lamps, including Model UV712, UV708, UV705, and UV702 (Trojan Technologies Inc., London, Ontario, Canada). UV0 was taken as the control. FT – feeding tank. The direction of the nutrient solution flow indicated with arrows.

Fertigation schedule and nutrient feeding formula were set up as in a routine tomato nutrient film technique (NFT) system, except extra solutions containing iron chelate (ethylene-diamine-tetra-acetic acid ferric monosodium, Fe-EDTA) (BDH chemicals Ltd., UK) were added to maintain a concentration of 1–2 mg Fe L^{-1} in the nutrient solutions. The concentration of iron was analyzed by an atomic absorption spectrophotometer (acetylene flame).

Plant cultivation and root rot assay

A tomato cultivar (*Lycopersicon esculentum* Mill. cv. Trust), susceptible to *P. aphanidermatum*, was used. Seedlings were grown on rockwool cubes for 42 days before planting. Tomato plants were grown for 138 days in a NFT system. Tomato fruits were harvested and recorded. Root rot severity was rated by a scale in which 1 = less than 10% root rot, 2 = 10–50% root rot, 3 = 50–80% root rot, 4 = more than 80% root rot.

Pythium *inoculum and survival*

Zoospores of *P. aphanidermatum* were produced as described previously (Zhang and Tu, 1999). One litre of the suspension of *Pythium* zoospores (2.65×10^4 CFU mL^{-1}) was added to 1400 L of solution

in the main feeding tank resulting in a density of 12 ± 3 CFU mL^{-1}. Samples of nutrient solution were collected at the inlet and the outlet of each row immediately after initial introduction of *Pythium*, and thereafter, at the outlet once a week. The solutions were vortexed thoroughly, and then 1 mL of the solution was plated onto a *Pythium*-selective medium (Burr and Stanghellini, 1973). The remaining solutions were used for bacterial enumeration.

Bacterial populations

Bacterial populations in the solution and rhizosphere were monitored weekly. One millilitre of the solution collected at each outlet of a row was diluted in a series of 0.1 M phosphate buffer (pH 6.8). An aliquot (0.1 mL) of the dilution was then plated on Difco tryptic soy agar (TSA). Colonies were counted after 48 h of incubation at 26 °C, and expressed as CFU mL^{-1}.

The first set of root samples was obtained 14 days after planting, and the second set at the end of the experiment. Segments of 2-cm-length root tips, of 1 g from six plants in the same row, were pooled and homogenized in 10 mL of phosphate buffer for 10 min. The suspension was serially diluted and immediately plated on TSA. The plates were incubated at 26 °C for 48 h, and then colonies were counted and expressed as CFU mL^{-1}.

Statistical analysis

All data were subjected to analysis of variance (ANOVA). When a significant ($P < 0.05$) F test was obtained for treatments, separation of means was accomplished by the least significant difference test ($LSD_{0.05}$). Data for microbial densities were log-transformed prior to analysis of variance. We checked homogeneity of variances and there were no differences among root rot tests. There were differences in variances when testing for fruit yield with different growth periods. However, the treatment trends among trials were similar and the differences were negligible, thereby we pooled the data to simplify statistical analysis. The experiment was repeated twice.

Results

Pythium *survival*

Following the introduction of *Pythium* inoculum, solutions collected from the outlet of the control contained 4.9 ± 1.2 CFU mL^{-1} of *Pythium*. In the UV-treated solutions, *Pythium* propagules were detected only at the inlet of UV19 (1.7 ± 0.1 CFU mL^{-1}) at the beginning, but were detected 14 days later in UV38, UV59, and UV88 (Figure 2). In the non-inoculated solutions,

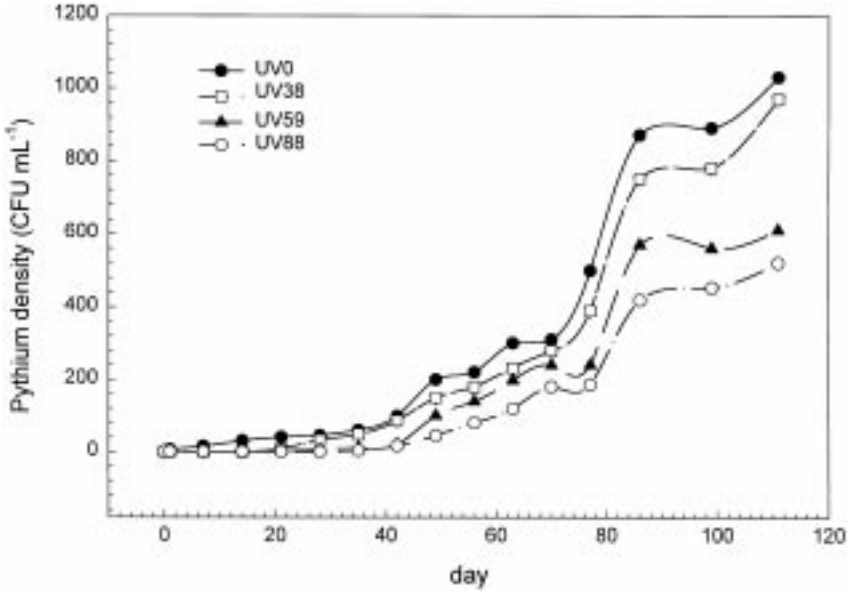

Figure 2. Population dynamics of *Pythium aphanidermatum* in recirculating solutions treated with different UV doses. UV0 as the control.

Pythium propagules were not detected in 50 days after planting. Thereafter, the number of *Pythium* propagules that survived different UV doses accumulated in the root systems as the solutions circulated through. The lower the UV dose the greater the accumulation of *Pythium*. *Pythium* populations grew fastest in the control compared to the others, and the lag phase of *Pythium* population growth was also shortest. The lag phases for the others extended concomitance with the increase of UV doses (Figure 2). For example, 49 days after inoculation, the population densities were 200, 150, 100, and 45 CFU mL^{-1} in the control, UV38, UV59, and 88 mJ cm^{-2}, respectively (Figure 2). There was no significant difference ($P < 0.05$) in *Pythium* survival between the control and UV19. At the highest dose, the exponential growth of *Pythium* populations occurred 70 days after infection. In the control, however, *Pythium* populations had been raised above 300 CFU mL^{-1} 70 days after the infection, and reached the highest population density of 1030 CFU mL^{-1} at the end of the experiment (Figure 2). In UV59 and UV88, *Pythium* populations still was less than 600 CFU mL^{-1}.

Bacterial populations

The bacterial density in the recirculating solution was 1.7×10^5 CFU mL^{-1} before subjecting to UV irradiation. The irradiation significantly ($P < 0.05$) reduced the populations in recirculating solutions. Seventy days into the experiment, the bacterial density decreased to 6.6×10^4 CFU mL^{-1} in UV88, while it remained more than 10^5 CFU mL^{-1} in the control (Figure 3). In contrast with the increase of *Pythium* populations, the bacterial populations continuously declined. At the end of the experiment, the bacterial density was only 3.63×10^3 CFU mL^{-1} in UV88. The bacterial populations also decreased in the control (UV0) as the age of the plant advanced although at a lower rate (Figure 3).

In the rhizosphere, the bacterial density was 6.77×10^9 CFU mg^{-1} fresh roots up until 14 days after planting, regardless of the treatments (Figure 4). After this time, the UV treatments suppressed population growth in the recirculating solutions significantly ($P < 0.05$). The populations in the rhizosphere was 2.53×10^9 CFU mg^{-1} fresh roots in the control at the end of the experiment while the populations in the UV38 and UV59 treatments decreased to 9.93×10^8 and 7.33×10^7 CFU mg^{-1} fresh roots, respectively (Figure 4). The biggest decrease in populations occurred for UV88 treatment where the recirculating solution was almost completely pasteurized. The populations dropped to 3.51×10^7 CFU mg^{-1} fresh roots at the end of the experiment (Figure 4). The rhizobacterial

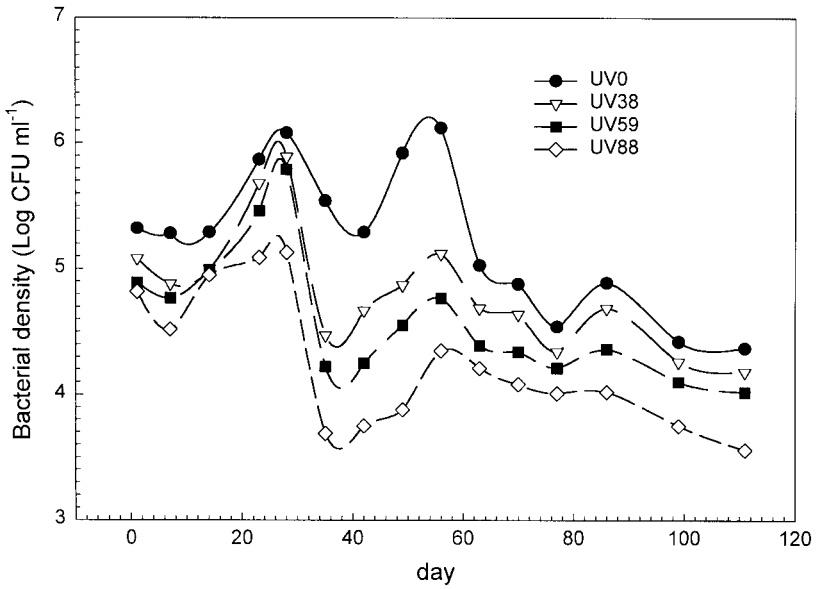

Figure 3. Population dynamics of bacteria in recirculating solutions treated with different UV doses. UV0 as the control.

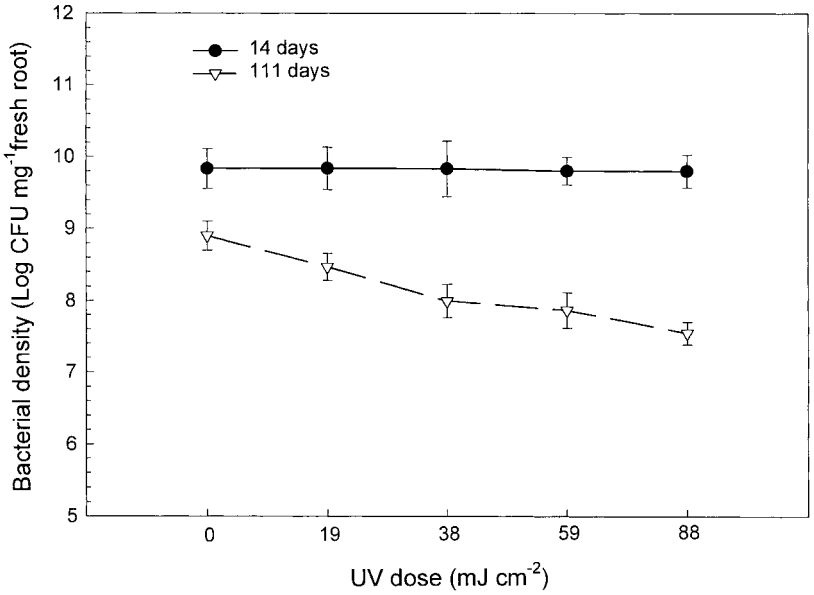

Figure 4. Kinetics of the bacterial density (Log CFU mg^{-1} fresh roots) in the tomato rhizosphere 14 and 111 days after treatment with different UV doses. Vertical bars represent the standard error, $n = 4$.

populations were significantly ($P < 0.05$) lower in the UV treatments than that in the control at the end of the experiment (Figure 4).

Disease incidence and fruit yield

Pythium root rot was observed in all treatments. There was little difference in root rot severity between the control and the UV treatments (Figure 5). Although the disease incidence varied among the UV treatments, root rot severity did not decrease when the UV dose increased. Apparently, the decrease in *Pythium* populations did not lead to concomitant decrease of root rot severity. Fruit yield data did not show any significant difference between the control and UV treatments.

Discussion

Procedures used for the disinfection of bottled water and wastewater were adapted to greenhouse hydroponics for the control of root pathogens (Runia, 1995). There is little doubt about the germicidal effect of UV irradiation if a sufficient dose reaches the target pathogen. Therefore, in theory, a recirculating solution can be disinfected to any degree by manipulating its dosage. In a reality, however, total disinfection of recirculating solution in a hydroponic system is extremely difficult to achieve. For example, *Pythium* propagules, especially oospores which more likely survived in UV irradiation, and then retain, multiply, and accumulate in rhizosphere. At a high UV dose, the accumulation of survived propagules may be minimal in a short period. Stanghellini et al. (1984) showed that spinach root rot caused by *P. aphanidermatum* is controlled by UV irradiation in a two-week trial at 90 mJ cm^{-2}. In a long growth season, however, the accumulation of survived propagules could be significant to cause root rot, especially when the non-target bacterial populations start to decline in rhizosphere due to the irradiation as indicated in previous study (Buyanovsky et al., 1981). In this study, tomato root rot caused by *P. aphanidermatum* was not reduced by UV irradiation, although a decline of *Pythium* populations did occur. The reduction from the initial pathogen density might not be great enough to eliminate or at least control of the disease. The low pathogen densities in this study may be enough to cause significant root rot in such environment. For example, in field soils, 70–89% of embryos were infected with *Pythium* spp. during germination of wheat seeds at 150–200 propagules g^{-1} soil. In pasteurized soils, however, embryo infection reached almost 100% with only 50 propagules g^{-1} soil (Fukui et al., 1994). In a recirculating nutrient film

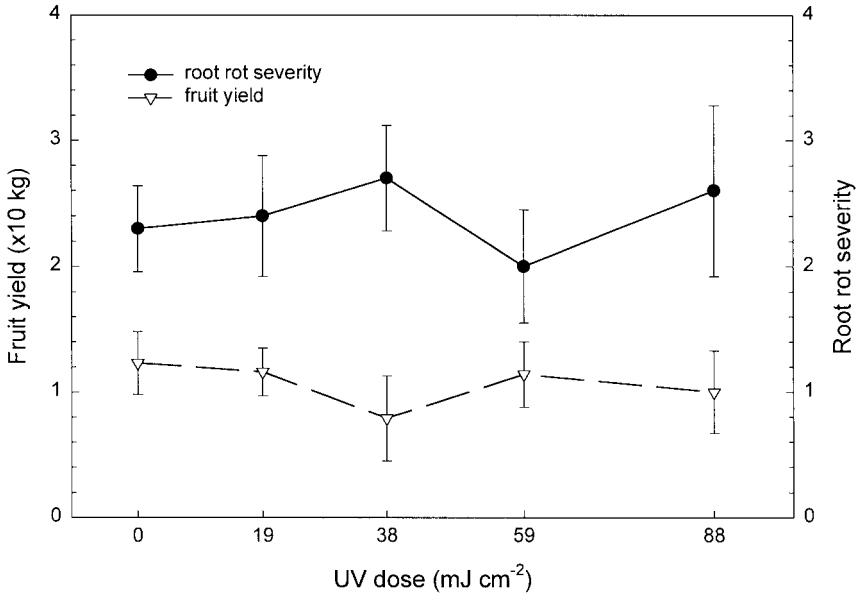

Figure 5. Tomato fruit yield and *Pythium* root rot in the treatment of different UV doses. Vertical bars represent the standard error, $n = 4$.

hydroponics, *Pythium* infection occurs on cucumber roots at as low as 0.22 CFU 100 L^{-1} of nutrient solution in a 28-day experiment (Menzies et al., 1996). Severe infection of tomato plants was observed at 200–300 propagules of *P. aphanidermatum* mL^{-1} nutrient solution in this study.

Some rhizobacteria provide a front-line defence for roots against pathogen attack in field soils, and play a key role in suppression of root pathogens in hydroponic cultures as well (Berger et al., 1996; Rankin and Paulitz, 1994; Paulitz et al., 1992; Paulitz, 1997). They can act as antagonists or nutrient competitors to inhibit *Pythium* growth and proliferation in the rhizosphere (Loper and Buyer, 1991). Rhizobacteria reduce colonization of root pathogens, such as *P. aphanidermatum* on cucumber roots, and *Pythium* root rot in closed rockwool systems (Paulitz, 1997). *Pseudomonas* spp. may compete for microsites and exudates with *P. aphanidermatum*, change the distribution of encysted zoospores on cucumber root surface, and induce systemic resistance in cucumber plants (Chen et al., 1998). Studies conducted at the University of Arizona imply that biosurfactants may play a role in reducing *Pythium* zoospore dissemination in hydroponic solutions by certain bacterial strains (Stanghellini and Miller, 1997). Inhibition of *Pythium* mycelium growth and zoospore germination by a number of bacterial isolates was observed in our previous study (Zhang and Tu, 1999). In this study, it was shown that the high dose of UV irradiation reduced bacterial density. The exponential growth of *Pythium* populations coincided with a significantly decline of bacterial populations in the recirculating solution although *Pythium* populations built up slowly in UV-treated solutions at the beginning. The decline of the populations of bacteria would open more spaces for the pathogen colonization and favours its establishment on the root surface. *Pythium* populations were suppressed in the high dose of UV treatments (i.e., UV88, UV59) in the two-month period of the experiment. With longer times, the density of *P. aphanidermatum* increased, while bacterial populations decreased in the high dose of UV treatments. Similarly, in pasteurized soils, the incidence of tomato root rot caused by the low *Pythium* population density in UV-treated nutrient solutions can be as high as in non-UV-treated solutions. As a result, UV treatments failed to reduce root rot severity. This observation is consistent with others (Berger et al., 1996) who suggest that the ratio of *Pythium* to non-target bacteria in total microbial populations is more important than the absolute numbers in causing of root rot under certain circumstances.

With acreage of greenhouse hydroponic tomatoes increasing in southern Ontario, yield loss caused by root pathogens has become significant. Root rot is severe during summer when the temperature reaches

to 28–32 °C in greenhouses. *Pythium* spp. are major pathogens in tomato root disease complex. At such high temperatures, *P. aphanidermatum* is the dominant species in all our *Pythium* isolates (unpublished data). Mobile zoospores of *P. aphanidermatum* are easily spread through recirculating nutrient solutions. Technology for disinfection of the recirculating nutrient solutions is highly desirable by greenhouse industry. However, the inconsistency of UV treatment in hydroponic cultures has limited its commercial application (Menzies et al., 1996). This inconsistency is unacceptable and requires more in depth research. The lack of relation between the decline of the pathogen density by UV disinfection and the control of root rot shown in this study may be one of the causes underlying such inconsistency. More research is required to analyse non-target bacterial populations affected by UV disinfection and to evaluate their implication in the lack of efficacy of the UV control.

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