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Effect of Solarization Intensity on the Control of Pink Root of Chives, and the Response of the Crop to AM Fungal Application

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Abstract

Two field experiments were conducted in the same farm over two consecutive years (1999 and 2000), to study the effect of soil solarization intensity on the establishment of arbuscular mycorrhizal (AM) symbiosis, and on the ability of AM fungi to control pink root disease induced by *Pyrenochaeta terrestris* and to improve the product yield. In both years, growth retardation was directly correlated with chemical fumigation in chive seedlings grown in solarized soil but inversely correlated with solarization intensity. Furthermore, the efficacy of controlling pink root disease in chive roots was increased by increased solarization intensity. Inoculation of chive with a *Glomus intraradices*-based inoculant reduced the phenomenon of growth retardation, induced plant crop productivity and resulted in a further decrease in pink root pathogenicity as compared with that in untreated plots. The results show that AM symbiosis can suppress pink root disease in chive under field conditions.

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1. Introduction

The potential of arbuscular mycorrhizal (AM) fungi to contribute positively to plant growth per se, has been well established (Smith and Read, 1997). On the other hand, infections with soil-borne pathogenic microorganisms have a negative impact on the growth and plant yield. The improved vigor of plants that were pre-colonized by AM fungi has been shown to protect against these pathogens (Borowicz, 2001). However, some studies have found AM fungal symbiosis to play only a minor or less effective role in this protection, but this could be related to differences among symbiont species.

Agricultural practices commonly include methods for controlling pathologenic soil microorganisms such as the fungus *Pyrenochaeta terrestris*, the causative agent of the pink root disease, which decreases crop productivity, especially during the later stages of the growing season. The common procedures for combating this pathogen include chemical intervention, with chemicals that include methyl bromide, metam sodium, and Dazomet™ (BASF, Germany). These materials, although they are very effective, are also highly detrimental, primarily because the chemical moiety is liable to be carried forward in the crop (leaves) and thus into the food chain, and also to form breakdown products that cause severe long-term environmental damage. In light of the current regulatory guidelines that ban the use of these chemicals in agriculture by the year 2004, alternative technologies for managing soil-borne pests are being actively explored; these include soil solarization, of which the efficacy, in comparison with chemical fumigants, has been encouraging.

Soil solarization is the process of heating soil by absorbing solar energy under clear plastic (Katan et al., 1980; Katan, 1981; Katan, 1981; DeVay and Katan, 1991; Mahrer, 1991); this reduces the indigenous microorganisms, both the pathogenic and the beneficial ones (Greenberger et al., 1987; Pinkerton et al., 2000). Solarization affects soil-borne plant pathogens at soil depths of up to 40–60 cm (DeVay and Katan, 1991). In Israel and other hot countries in the Middle East, soil solarization is commonly used in onion and chive fields to control diseases such as pink root – caused by *Pyrenochaeta terrestris* and *Fusarium* – and also nematodes and other soil organisms (Katan et al., 1980; Rabinowitch et al., 1981; Satour et al., 1989; Chen et al., 1991). Soil solarization often results in increased amounts of soluble nutrient such as N, P, K and microelements (Chen et al., 1991). These changes together with microbial changes following solarization lead in certain cases to an increase growth response phenomenon of plants (Gamliel and Katan, 1991).

On the other hand, because of their non-discriminatory nature, both chemical fumigation and soil solarization also reduce the populations of beneficial indigenous microorganisms, such as AM fungi. Thus, this practice tends to induce early growth retardation, leading to reduced yield. In our previous studies, we showed that this early growth retardation could be overcome by applying AM fungi to the plant (Wininger et al., 2003; Bendavid-Val et al., 1997).

Wininger et al. (2003) demonstrated that AM fungal application could support the early stages of plant adaptation after transplanting, and so ensure a superior early round of harvesting. The present study seeks to optimize the efficacy of soil solarization as a sole means of biocontrol, and to test its ability to control the pink root disease. We also evaluated the effect of AM fungal colonization on chive productivity during the later stages of the growing season – a period when the plant is susceptible to re-infection by the pink root disease.

2. Materials and Methods

Field experimental details

Two field experiments were conducted during 1999–2001 in two commercial greenhouses located in the Yavneel Valley area (north-eastern Israel). This was done to allow a direct comparison between seasons, and with our previous work (Wininger et al., 2003). For convenience, the experimental procedures carried out during 1999 and 2000 are referred to as "Experiment I" and "Experiment II", respectively and the duration span of each is depicted in Fig. 1. The harvests for Experiment I and Experiment II were carried out over six and four consecutive rounds of harvesting, respectively. The experiments were farmed within the whole production system of the farmer, under the common and recommended practices.

The soil characteristics of Yavneel were as follows: clay with 0.51% organic matter; 34% clay, 46% silt and 20% sand; pH 8.2. A chive crop had been cultivated in the site prior to the initiation of the present experiments. This site had a long history of pink root infection by *P. terrestris*, which reduced the chive productivity, especially in the later rounds of harvesting, which are commercially very important for this crop.

Experimental design and soil solarization procedure

A factorial experimental design was employed, to study the effects of solarization intensity and of AM fungal inoculations on chive development, pink root control, and crop yield.

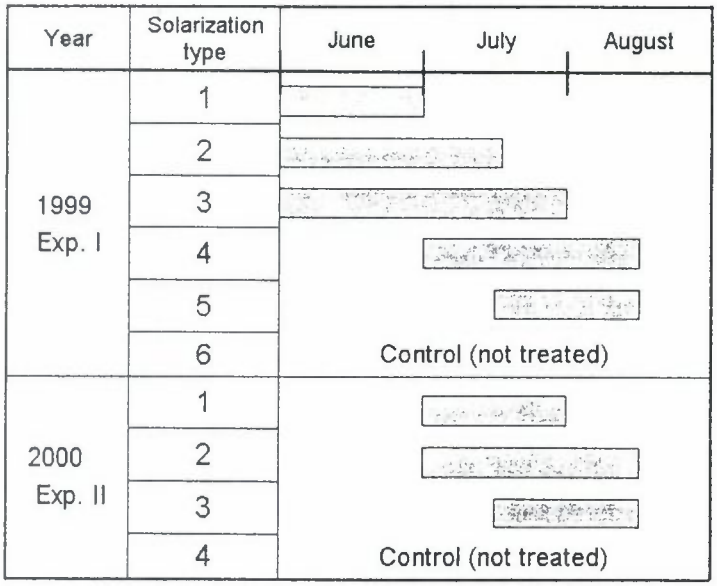

Figure 1. Scheme of timing and duration of soil solarization treatment in Yavneel Valley carried out during 1999 and 2000, as described in the Materials and Methods section.

Plots were arranged in a randomized block and split-plot design with four replications per treatment, each comprising four adjacent beds (10 m long × 6–8 m wide). Solarization was carried out by mulching the cultivated wet soil with clear, 0.035-mm-thick, polyethylene sheets (Genegar, Israel). The daily temperatures readings of the solarized soil in both years are presented in Fig. 2. Soil temperature data were continuously collected by a micrologger (CR-21X, Campbell Scientific Inc., Logan, Utah), throughout both experimental periods.

Plant material and AM inoculum

Chive (*Allium schoenoprasum*) cv. "Denfeld" was used in both experiments. The AM fungus, *Glomus intraradices* [Schenck & Smith], isolate LPA8, was cultivated in the Volcani Center in Bet Dagan, in association with sorghum (*Sorghum bicolor* L.) as host. The inoculum consisted of spores, hyphae and infected roots, and it was introduced into the growth medium by mixing it with the commercial potting mix (peat moss: vermiculite at 7:3, v:v). Inoculum was incorporated in the growth medium at a rate of 10% (v/v), in the nursery before the plants were transplanted into the greenhouse. The chives were sown in the inoculated potting mix, in which they grew for 4 weeks before being transplanted to the fields, according to the experimental design.

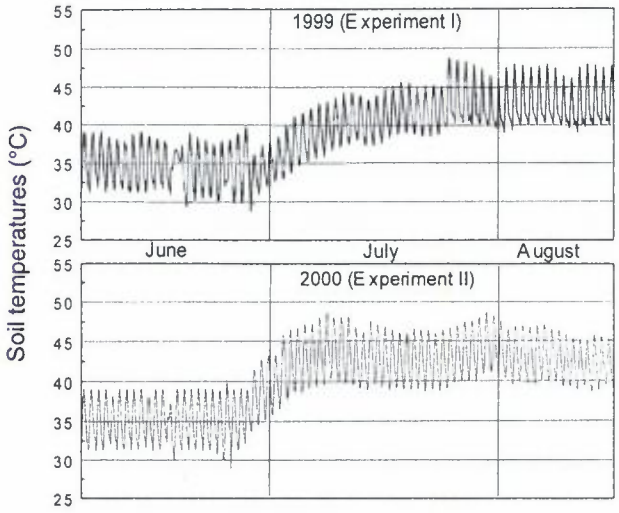

Figure 2. Daily soil temperature, which were measured during the whole solarization process in Yavneel experiments. Values were taken at a depth of 20 cm, under clear polyethylene mulch during the summers of 1999 (experiment I) and 2000 (experiment II).

Assessment of root AM inoculation and pink root disease

Plants from each plot were sampled periodically, at the commercial harvest time. Samples were taken from each treatment along 1 m of row (0.9 m^2). The root system was rinsed and rated for external pink root symptoms, i.e., root rot characterized by a distinctive pink-purple discoloration of the roots. Pink-root severity was measured on an index scale of 0–3, where 0 = clean white roots with no symptoms, and 3 = root system completely covered with pink root symptoms. Infected roots were taken to the laboratory in order to verify the presence of *Pyrenochaeta terrestris* by isolating the fungus on an appropriate agar medium (Katan et al., 1980).

At harvest time leaves were cut 4 cm above the soil surface within an area of 100 cm^2. Leaves were weighed to determine their fresh weight and were graded as export quality or non-marketable, according to the commercial standards of leaf length and appearance. When samples included root systems to be evaluated for AM colonization, the entire root systems of 10 randomly chosen plants within the sampled area were pooled, washed, cleared and then stained with trypan blue solution (Phillips and Hayman, 1970). Assessment for mycorrhizal colonization was carried out under a dissecting microscope, and percentage colonization was estimated by using the gridline intersection method according to Giovannetti and Mosse (1980).

Statistical analyses

Data were first subjected to analysis of variance (ANOVA), to detect possible interactions among the main factors (solarization, AM inoculation), and then to Student's t-test to separate the means. Data presented as percentages were transformed to arcsine values before analysis. The disease index was transformed and analyzed by the rank procedure. All analyses were performed at $P<0.05$, with SAS software, release 8.0 for PC (SAS Institute Inc., Cary, NC).

3. Results

Recording of soil temperature data

In both years (1999 and 2000) the soil temperature increased during July, and was maintained at elevated levels through August. The temperature variation patterns were similar in the two years, but the temperatures in July 2000 were higher than those in July 1999. Following the solarization treatment of 1999, there was a severe reduction of the indigenous AM fungal population (Wininger et al., 2003), and this was observed in 2000 also.

Effect of AM fungal inoculation on plant development and growth in solarized soils

As we found previously (Wininger et al., 2003), during the 1999-growing season, the AM fungal inoculation affected chive growth under field conditions as early as the first and second rounds of harvesting. Furthermore, it was concluded from this experiment that in the Yavneel region, early solarization treatments during June are less effective than later (July and August) treatments. In light of this finding, the effects of solarization application during July and August were evaluated in Experiment II (Table 1).

During the early part of the growing season, plants with non-mycorrhitic roots grew more slowly than those with mycorrhitic ones, and necrotic and stunting responses were evident within two weeks from the date of transplantation. In Experiment II, the positive contribution of AM fungal inoculation was detected right from the first two harvests (Table 1). The greatest benefits of mycorrhization were obtained when soil that had been solarized from the beginning of July to the end of August was used for cultivation; the next greatest benefit was obtained by AM fungal treatment of soil that had been treated for a shorter period during July or from mid-July to mid-August. As can be inferred from Fig. 1, the highly elevated temperature

profiles during July and August, in both Experiment I and Experiment II, significantly affected the efficacy of soil solarization. It is interesting to note that during the second harvest, when the effect of mycorrhization was significantly greater in mycorrhitic than in non-mycorrhitic plants, all solarized treatments resulted in significantly higher yields than those obtained in non-solarized soils.

Table 1. Effects of soil solarization intensity and duration of solarization types on chive fresh weight in the presence (+AM) and absence (–AM) of mycorrhizal inoculants. Results were obtained from 1st and 2nd harvests in the Yavneel experiment in 2001. Values are means of four replicated plots and different letters within main effect denote significant differences ($P<0.05$).

Solarization type*	Harvest 1			Harvest 2		
	–AM (g/m^2)	+ AM (g/m^2)	Average (g/m^2)	–AM (g/m^2)	+ AM (g/m^2)	Average (g/m^2)
1	584	846	715 b	1923	2053	1988 a
2	540	855	696 b	1869	2109	1989 a
3	702	957	829 a	1966	2129	2047 a
4	759	898	828 a	1545	1632	1589 b
Average	646 b	889 a		1825 b	1980 a	

*Solarization types are described in Fig. 1.

Table 2. Effect of soil solarization intensity and duration of solarization types on pink root incidence and severity in chive plants in the presence (+AM) and absence (–AM) of mycorrhizal inoculants in the Yavneel experiment in 1999. Pink root was determined in April 2000. Values are means of four replicated plots, and different letters within main effects denote significant differences ($P<0.05$).

Solarization type*	Incidence (%)			Severity (0–3)		
	–AM	+ AM	Average	–AM	+ AM	Average
1	53	21	37 b	0.7	0.3	0.50 b
2	34	7	20 bc	0.4	0	0.20 c
3	20	8	14 c	0.1	0	0.05 c
4	4	0	2 d	0.2	0	0.10 c
5	3	4	4 d	0.3	0	0.15 c
6	100	83	92 a	1.4	1.3	1.35 a
Average	36 a	20 b		0.51 a	0.26 b	

*Solarization types are described in Fig. 1.

Table 3. Effect of soil solarization intensity and duration of solarization types on pink root incidence and severity in chive plants in the presence (+AM) and absence (–AM) of mycorrhizal inoculants in the Yavneel experiment in 2000. Pink root was determined in April 2001. Values are means of four replicated plots and different letters within main effect denote significant differences ($P<0.05$).

Solarization type*	Incidence (%)			Severity (0–3)		
	–AM	+ AM	Average	–AM	+ AM	Average
1	8.4	2.7	5.6 b	0	0.1	0.05 b
2	1.8	1.4	1.6 b	0	0	0 b
3	5.2	7.8	6.5 b	0.1	0.1	0.10 b
4	83.2	47.9	65.6 a	1.1	0.6	0.85 a
Average	24.7 a	14.9 b		0.3 a	0.2 a	

*Solarization types are described in Fig. 1.

Effects of solarization intensity and AM inoculation on pink root disease

Solarization intensity was positively correlated with the control of pink root disease in chive plants in both Experiment I and Experiment II. Solarization, which was continued from July through August, was most effective in controlling the disease, whereas the treatments that lasted from June to mid-July 1999 were only partially effective (Table 2). Solarization intensity as expressed by degree hours was significantly correlated with reduction of disease incidence (Fig. 3). The results presented in Fig. 3. clearly show that effective control of pink root disease is dependent on cumulative heat dosage (degree hours) and not necessarily the length of solarization. AM application reduced the severity of pink root incidence, and was most effective in nonsolarized plots and in solarization treatments that were carried out during June. AM fungi were able to reduced both disease incidence and severity.

Similar trends were observed in Experiment II, which was carried out during 2000. Although in this experiment the solarization treatment was applied during the most effective period for solarization – July and August – a significantly lower percentage incidence was obtained on mycorrhitic roots than on the non-mycorrhitic controls (Table 3). It is evident that AM fungal inoculation reduced the percentage incidence by 42% in non-solarized soil (controls) (Table 3). Also, the severity of the disease symptoms was reduced by 45% in mycorrhitic roots. However, all solarization treatments significantly reduced the percentage incidence as well as the severity of pink root disease (Tables 3).

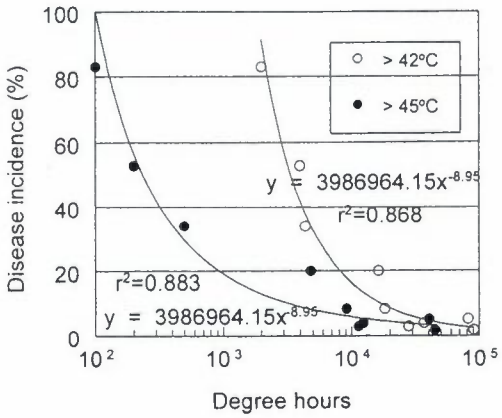

Figure 3. Relationship between solarization intensity and control of pink root disease. Solarization intensity was calculated as cumulative degree hours over 42°C and 45°C. Data was pooled for the two experiments. Degree hours were calculated from soil temperatures (Fig. 2). Pink root disease data was taken from Tables 2 and 3.

Effect of solarization intensity and AM fungal inoculation on yield

Significant increases in yield were obtained as a result of solarization in both experiments (Tables 4 and 5). The increase in yield resulting from solarization was proportional to the intensity of the solarization treatment, and was attributable to the effective control of the pink root disease. Although yield values differed between the seasons, solarization treatment in Experiment I, during July–August, resulted in 6–9% increases in export-quality and total fresh leaves. In Experiment II, during the same season (July–August), solarization treatment increased the export-quality and total yields by 31–45% and 22–25%, respectively. In summary, the AM fungal application improved total and export-grade yields in both experiments (I and II), albeit at very different rates.

4. Discussion

AM fungal application reduced the severity and percentage incidence of pink root disease in chive roots. This reduction was more pronounced when solarization were not used and disease incidence was high. This inhibition in plant growth was related to the fact that natural symbiosis with AM fungi was prevented. It was only when plants were colonized with AM fungus that a higher crop growth potential could be obtained (Tables 4 and 5.).

Table 4. Effect of soil solarization intensity and duration of solarization types on chive fresh weight in the presence (+AM) and absence (–AM) of mycorrhizal inoculants. Results express the yields of six harvests during November 1999 – May 2000 and were graded as export-quality or non-marketable grades. Values are means of four replicated plots and different letters within main effect denote significant differences ($P<0.05$).

Solarization type*	Export			Non-marketable		
	–AM (kg/m^2)	+ AM (kg/m^2)	Average (kg/m^2)	–AM (kg/m^2)	+ AM (kg/m^2)	Average (kg/m^2)
1	3.05	3.20	3.13 c	3.35	3.76	3.55 ab
2	2.96	3.23	3.09 c	3.43	3.89	3.66 a
3	2.94	3.54	3.24 b	3.44	3.82	3.63 a
4	3.13	3.55	3.34 ab	3.55	3.82	3.68 a
5	3.30	3.59	3.45 a	3.38	3.88	3.63 ab
6	2.98	3.30	3.14 c	3.29	3.65	3.47 b
Average	3.06b	3.40 a		3.40 b	3.67a	

Solarization type*	Total		
	–AM (kg/m^2)	+ AM (kg/m^2)	Average (kg/m^2)
1	6.40	6.96	6.68 b
2	6.34	7.12	6.75 b
3	6.38	7.37	6.87 ab
4	6.68	7.37	7.02 a
5	6.97	7.47	7.22 a
6	6.26	6.95	6.61 b
Average	6.51 b	7.15 a	

* Solarization types are described in Fig. 1.

In a previous study we demonstrated that productivity of plants was most affected in the early stages of growth (Wininger et al., 2003), but in the present study we demonstrated that AM inoculation on roots that were not colonized by native AM fungi could affect the final yield of the crop.

With few exceptions, crop plants have AM fungal associations, but the extent of root colonization by AM fungi and the effects of symbiosis on a particular crop may vary, depending upon the environment in which the association is manifested. In most cases, AM fungi significantly change the pattern of root exudation, and the microbial composition of the soil in the mycorrhizosphere.

Table 5. Effect of soil solarization intensity and duration of solarization types on chive fresh weight in the presence (+AM) and absence (–AM) of mycorrhizal inoculants. Results express the yields of four harvests during November 2000 – May 2001 and were graded as export quality or non-marketable grade. Values are mean of four replicated plots and different letters within main effect denote significant differences ($P<0.05$).

Solarization type*	Export			Non-marketable		
	–AM (g/m^2)	+ AM (g/m^2)	Average (g/m^2)	–AM (g/m^2)	+ AM (g/m^2)	Average (g/m^2)
1	1385	1618	1500 ab	2572	2792	2682 a
2	1384	1557	1470 b	2539	3088	2813 a
3	1556	1713	1634 a	2600	2967	2648 a
4	1139	1105	1122 c	2187	2414	2300 b
Average	1366 a	1498 a		2474 b	2815 a	

Solarization type*	Total		
	–AM (g/m^2)	+ AM (g/m^2)	Average (g/m^2)
1	3957	4410	4183 a
2	3923	4645	4284 a
3	4156	4313	4234 a
4	3326	3519	3422 b
Average	3840 b	4221 a	

* Solarization types are described in Fig. 1.

These changes could greatly influence the growth and health of plants, in part through the biological suppression of plant diseases. Disease suppression may be the result of reduction of environmental factors that could limit plant growth and predispose the plants to infection by opportunistic pathogens. More important, however, are the specific morphological and physiological changes that directly or indirectly result in lower incidence and/or severity of plant diseases in AM plants than in non-AM plants. The effects of AM fungi on pathogens are most likely indirect, and result from improved nutrition or altered physiology of the host (Dehne, 1982). Most commonly, AM fungi appear to enhance host tolerance by improving root growth and function (Hussey and Roncadori, 1982; Smith, 1988). AM fungi may also increase host resistance (reduce pathogen performance) by stimulating the defense response (Volpin et

al., 1994; Morandi, 1996) or by altering the root exudation pattern (Graham and Menge, 1982; Pinior et al., 1999). AM fungi are also hypothesized to suppress pathogen growth by competing with pathogens for infection sites or photosynthates (Smith, 1988; Muchovej et al., 1991), or by promoting the growth of soil microbes that are antagonistic to the pathogens (Linderman, 1992).

Soil solarization was developed primarily for controlling soil-borne pathogens and weeds (Lifshitz et al., 1983; Freeman and Katan, 1988; Katan and DeVay, 1991; Gamliel and Katan, 1991), but quite often, a positive increase in the plant growth response has been observed (Stapleton and DeVay, 1984; Gamliel and Katan, 1991; Gruenzweig et al., 1993; Wininger et al., 2003). It has been demonstrated that after 4 weeks of solarization treatment, the AM fungal population in the soil declined significantly at depths of 0–40 cm (Bendavid-Val et al., 1997). In the case of ectomycorrhizal fungi, this treatment eliminated these symbiotic organisms from the forest nursery, to a depth of 15 cm in the soil profile (Soulas et al., 1997).

Bendavid-Val et al. (1997), found a significant decrease in numbers of AM fungal propagules and in mycorrhizal colonization of onion and carrot roots growing in solarized soils; root colonization was not evident until 6 weeks after transplanting the plants in treated soil. Moreover, Bendavid-Val et al. (1997) found that the reduction in the AM fungal population was associated with plant growth retardation in onion and carrot, the roots of which have a shallow spread compared with that of a crop with a deep root system, such as wheat. Pullman et al. (1981) found that solarizing the soil caused a decrease in AM fungus propagules at one site, but not at another; the reduction of AM fungi was associated with the attainment of higher soil temperatures in the solarized plots at the first site than in those at the second site. Other studies have found either no effects or even increased AM colonization, in solarized soils that reached similar temperatures to those which resulted in suppression of AM fungi elsewhere (Afek et al., 1991; Nair et al., 1990).

Previous attempts to control pink root used chemical means (e.g., methyl bromide), which had a long persistence in the soil and also caused stunting of the plants (Menge, 1982; Trappe et al., 1984). Furthermore, the deleterious effects of the chemicals were extended because of the persistence of degradation products in the soil, and through products being rendered unsuitable for export by carryover of chemical residues. In our present study, the pink root disease affected a large proportion of the root system when the soil was not treated with solarization. However, in solarized soil without AM fungal application, low yields were recorded because of the destruction of the native AM fungal population, therefore, in the present study, pre-inoculation of the chive crop was found to be imperative. On the other hand, there are only rare cases the AM increased incidence of root diseases. As the use of chemical fumigants is to

be phased out of commercial agriculture, the use of soil solarization and AM fungal inoculation could be a means to achieve more environment-friendly and sustainable agricultural practices.

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