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Using Entomopathogenic Nematodes to Control Insects During Stand Establishment

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I. INTRODUCTION

Using entomopathogenic nematodes as biological control agents is not a new idea. One of the earliest published accounts of a field study using reared nematodes was published in 1935, using *Steinernema glaseri* (Steiner) to control Japanese beetle *(Popillia japonica* Newman) grubs in soil (Glaser and Farrell, 1935). Recent advances in the development of large-scale, in vitro rearing techniques and formulation technology have prompted the development and commercialization of these nematodes as biological pesticides. Indeed, the potential for these nematodes as biological control agents is very promising, with proven efficacy against a variety of soil-inhabiting insects, including root weevils (several genera) (Jansson et al., 1990; Schroeder, 1987, 1990), white grubs *(Phyllophaga* spp.) (Kard et al., 1988), mole crickets *(Scapteriscus* spp.) (Parkman et al., 1993), fleas *(Ctenocephalides* spp.) (Henderson et al., 1995; Manweiler ,1994), and fungus gnats *(Bradysia* spp.) (Harris et al., 1995). In addition, under certain conditions, efficacy has been demonstrated against some foliage-feeding insects (Begley, 1990).

The technology for production, storage, and formulation of entomopathogenic nematodes has improved dramatically within the past 10 years. Although nematodes are easily produced in vivo, the high costs of in vivo production make it unsuitable for commercial use. A cost-effective in vitro production technique has been developed and successfully adapted to several species of steinernematids (Friedman et al., 1989). Steinernematid nematodes are now produced consistently in 30,000 to 80,000-L liquid fermenters in a proprietary medium

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with yields of up to 150,000 infective juveniles/mL. This efficient production technique has resulted in steinernematid nematode products that can compete economically with many chemical insecticide products. At this time, heterorhabditid nematodes cannot be produced efficiently in liquid culture, but can be produced in a less cost-effective solid medium (Hominick and Reid, 1990), resulting in products that are three to 10 times more expensive than steinernematid products.

II. NEMATODE MODE OF ACTION AND HOST SPECIFICITY

Nematodes in the families Steinernematidae and Heterorhabditidae are obligate parasites of insects. They have a symbiotic relationship with the bacteria, *Xenorhabdus* spp., which plays a critical role in the life of the nematode. The infective stage of the nematode is the third stage juvenile, which is free-living, motile, and nonfeeding (Fig. 1). This is the only stage of the life cycle that can survive outside the host. When the infective juvenile locates a host, it enters the insect through any of the natural openings—mouth, anus, or spiracles.

Once inside the body cavity, the nematodes migrate to the hemocoel (where the insect's blood is located), where development begins. Once in the hemocoel, the bacteria are released by the nematode, where they begin to multiply, causing septicemic death of the insect, usually within 24 to 48 h. The proliferation of the bacteria creates an ideal environment for the growth and reproduction of the nematode. Developing nematodes feed on bacterial cells and host insect tissues. The nematode will generally pass through several generations within the insect cadaver, until third-stage infective juveniles emerge into the environment where they search for a new host. Although there is variability between nematode species and strains, the nematode generally completes its life cycle in 10 to 20 days at 18 to 28 °C (Fig. 2).

Steinernematid and heterorhabditid nematodes have been isolated from a variety of insect hosts. Laboratory tests indicate that large doses of nematodes can induce mortality in a broad range of hosts that include most insect orders. Occasionally, this mortality can extend into other invertebrate groups, for example, Arachnida, Symphyla, and Crustacea. However, due to behavioral and ecological barriers, the effective host range of the nematode is generally restricted to soil-dwelling insects (Gaugler, 1988). To date, these nematodes have been shown to have little detrimental effect on nontarget organisms (Georgis et al., 1991; Poinar, 1989).

In the United States, the Environmental Protection Agency (EPA) has exempted the nematode–bacterium complex from safety registration (Nickle et al., 1988). Individual states generally require a formal registration to sell a specific nematode product within that state, but this procedure is generally straightforward. Many other countries have accepted the declaration of the U.S. EPA, and have also exempted nematodes from registration, but some have certain restrictions. For example, prior to introduction in Japan, the governing body required additional toxicity testing of the nematode.

III. APPLICATION TECHNOLOGY

Nematodes can be applied to the target zone with most commercially available spray equipment, including small pressurized sprayers, mist blowers, fan sprayers, as well as aerial application via helicopters (Georgis, 1990). In addition, nematodes can be applied using many types of irrigation, including drip and sprinkler systems. Pressures of up to 2068 kPa have no detrimental effect on nematodes, although the upper pressure threshold is unknown. Nematodes have been destroyed in high pressure (13,790 kPa) turf injection sprayers (biosys, unpublished data). The nematodes (≈20 to 25 µm in width) can easily pass through sprayer screens with openings as small as 50 µm in diameter.

Pre- and posttreatment irrigation of the target zone is necessary for maximum efficacy of nematode-based products. Free water is required by the nematode for active movement, and also serves to carry the nematode into the soil profile to the target pest. Irrigation greatly enhances the persistence and pathogenicity of the nematode in the soil (Georgis and Gaugler, 1991; Shetlar et al., 1988). In general, at least 6 mm of pretreatment irrigation, and 6 to 12.5 mm of irrigation within 30 min of treatment are recommended (biosys, unpublished data). Moisture in the treated area should be maintained for several weeks after the nematode application, either by rainfall or irrigation.

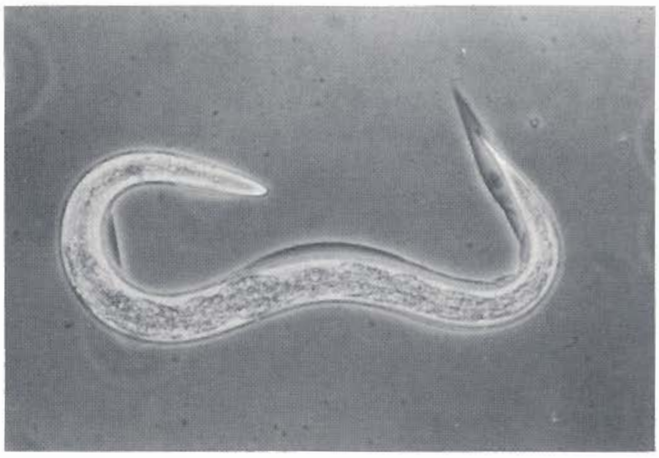

Fig. 1. Third-stage infective juvenile of the nematode, *Steinernema carpocapsae* (500 µm long, 20 µm wide).

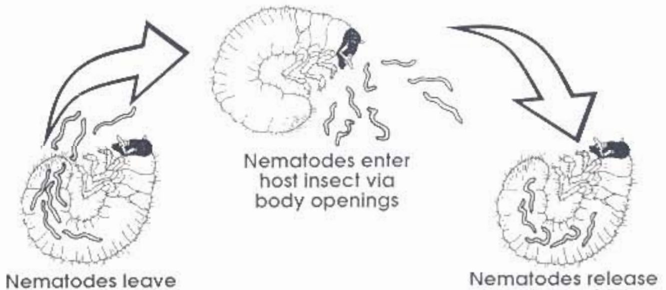

Fig. 2. Generalized life cycle of steinernematid and heterorhabditid nematodes.

Application of nematode-based products should be timed so that temperature extremes and exposure to intense ultraviolet irradiation are avoided. Nematode reproduction and pathogenicity can be affected by short-term (1.5 to 6 min) exposure to the most harmful wavelengths of ultraviolet radiation, and complete inactivation can occur after 5 to 10 min (Gaugler and Boush, 1978, Gaugler et al., 1992). In addition, high temperatures (>33 °C) can be detrimental to beneficial nematodes; thus, water used during mixing and application, and soil temperatures should remain below this threshold (Grewal et al., 1994). If nematodes are to be applied with existing irrigation equipment, lines should be flushed prior to nematode injection to ensure cool conditions throughout the system.

Integrating nematodes with other means of insect control is a strategy that holds promise (Kaya, 1985; Georgis, 1990). Nematodes can be tank-mixed with commercial formulations of *Bacillus thuringiensis* Berliner (Poinar et al., 1990), pyrethroids (Rovesti et al., 1988), and other pesticides and fertilizers (Georgis, 1990). Clearly, there are some pesticides that have detrimental effects on nematodes; however, they can still be used in an integrated system if certain precautions are taken. For example, waiting periods (0, 7, or 14 days) have been established for many commonly used agrochemicals (biosys, unpublished data). Nematode efficacy can be successfully augmented when used in combination with chemical pesticides (Forschler and Gardner, 1991; Quattlebaum, 1980) and other microbial agents (Barbercheck and Kaya, 1991).

IV. EXAMPLES OF NEMATODE USE DURING STAND ESTABLISHMENT

A. Citrus

The citrus root weevil complex is a group of insects considered to be major pests of citrus in Florida. The sugarcane rootstalk borer, *Diaprepes abbreviatus* (L), citrus root weevil, *Pachnaeus litus* (Germar), and northern citrus root weevil, *P. opalus* (Olivier) are widespread in Florida, while the little leaf notcher, *Artipus floridanus* (Horn.), and Fuller rose beetle, *Asynonychus godmani* (Crotch), are less common. Adult weevils of all species feed on the young, tender foliage, and heavy infestations can defoliate young trees. Larvae feed underground, and can cause severe damage to the root system, reducing tree vigor. *Diaprepes abbreviatus* larvae are particularly damaging to young plantings, and as few as one to two actively feeding larvae can kill a young tree in a short time (Schroeder and Sutton, 1977).

In 1994, the product BioVector® 355, containing the nematode *Steinernema riobravis* Cabanillas, Poinar & Raulston, was introduced into the citrus market, labeled for the control of *D. abbreviatus, P. litus,* and *P. opalus.* BioVector® 355 replaced an earlier product called Biovector, that contained the nematode *S. carpocapsae.* In five, replicated field trials, BioVector® 355 reduced weevil emergence by an average of almost 90% (Fig. 3). The product acceptance resulted in its appearance in the *Florida Citrus Spray Guide* (Univ. of Florida) (Knapp, 1995), indicating recognition as a university-tested pest control product. In 1994, more than 10,000 ha of grove and nursery citrus were treated with BioVector® 355 and is projected to reach 13,000 ha in 1995. BioVector® 355 has been successfully applied through commercial pumps and irrigation equipment. Injection of the product through microirrigation systems is the preferred application method, because of the reduced labor costs and the accuracy of product incorporation. Distribution of the nematodes in BioVector® 355 through microirrigation equipment is uniform when the system is functioning properly.

Stand establishment in citrus can occur under several circumstances. Replacement trees, which are planted in an established grove (resets), new grove plantings, nursery stock, and potted material being grown as ornamentals can all be considered plants in the stand establishment phase. Young citrus trees under these conditions are particularly susceptible to phytotoxic damage from fertilizers, herbicides, and insecticides. BioVector® 355 is pathogenic to all underground stages of the insects *D. abbreviatus, P. litus,* and *P. opalus.*

Planting guidelines for young citrus suggest that a basin be created around the newly planted tree, which is filled with water at planting. This "mudding-in" process is an ideal time to make the initial application of BioVector® 355 to protect the young tree against damaging grubs, which may already exist in the grove. The continued irrigation of the young tree will enhance the movement and survival of the nematode. Subsequent applications of BioVector® 355 can be made to the entire grove with existing spray or irrigation equipment.

Citrus grown as bare-root nursery stock ("liners") with its extremely tender foliage and roots, and a large number of trees in a small area make these plantings especially vulnerable to attack by citrus root weevils. A weevil grub can literally move down a row of liners, feeding on several plants in a short period of time. Again, this is an ideal situation for a nematode product—a highly managed, well-irrigated crop, with a susceptible target insect.

Containerized citrus trees are grown in Florida as resets and as

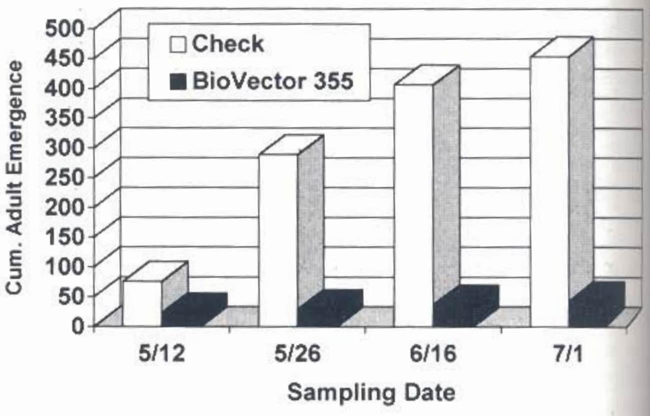

Fig. 3. Cumulative emergence of adult *Diaprepes abbreviatus* treated with BioVector 355 *(Steinernema riobravis)*, and from nontreated trees. Adult emergence based on ground cages (100 per treatment, 10 per replicate). Data from five trials conducted in 1994 in Vero Beach and Ft. Pierce, Fla. (Unpublished data; R.C. Bullock, Univ. of Florida, Ft. Pierce, Fla.).

ornamental plants. Weevil larvae can be extremely difficult to control in containers because they can easily move to hydrophobic areas inside the pot to avoid chemical pesticides. The application of chemical pesticides to potted citrus is also very expensive and labor-intensive. Because entomopathogenic nematodes actively search for their hosts, they can penetrate dry areas and locate the grubs. A detailed study of the efficacy of BioVector® 355, in which pots were broken open and weevil larvae counted, indicated a reduction in weevils of 94% over the nontreated control (biosys, unpublished data). The addition of a wetting agent (e.g., an organosilicone) to the nematode solution can enhance this efficacy by helping to wet the hydrophobic areas. Nematodes are easily applied to potted citrus by injection through overhead irrigation, or by hand gun directed into the pot.

B. Ornamentals

Ornamentals represent a unique challenge for insect control products because of the myriad of plant species, potting media, growing conditions, production techniques, and insect pests. In addition, tolerance to damage from insects and associated diseases is very low because it directly affects the marketability of the crop. The notion of effective biological control of ornamental pests is particularly attractive because of the many problems associated with chemical pesticides, including phytotoxicity, reentry periods, labor costs associated with frequent applications, selection for resistance in target pests, and the decreasing supply of effective, registered materials.

One group of insects commonly associated with greenhouse, nursery, and interiorscape plants are the fungus gnats, *Bradysia* spp. Fungus gnat larvae feed on plant roots and soilborne fungi. Infestations can significantly reduce root weight of seedlings (Kennedy, 1976) and have been implicated in the transmission of several disease organisms (Harris et al., 1994; Jarvis et al., 1993). In addition, adult fungus gnats can render plants unmarketable by depositing fecal material on the foliage, and are considered a nuisance pest by greenhouse workers.

During stand establishment, ornamental plants, which are often propagated via tissue culture, are "pushed" (overfertilized) to maximize growth. The plants are subjected to almost constant irrigation, which favors the buildup of fungus gnats. Further, because plants are regularly moved, depending on growth stage, fungus gnat infestations can be easily spread throughout a facility. During stand establishment, plants are particularly susceptible to damage to the root system and phytotoxicity from chemical pesticides.

The nematode *Steinernema feltiae* (Filipjev), is currently availab as the product X-Gnat® for the control of fungus gnat larvae. Th product is applied as a soil drench directly to the growing mediu raised beds, and underneath benches where fungus gnat larvae are found. The introduction of new plants into a treated area requires immediate treatment to prevent a reinfestation.

Control of fungus gnat larvae with X-Gnat® is comparable to, or better than, existing commercial products (Fig. 4). The product can be safely applied to all types of ornamental plants, and there are no reentry restrictions or protective clothing requirements for applicators. X-Gnat® can be applied using most conventional greenhouse sprayers or overhead irrigation equipment. Applications through drip irrigation or misting systems are not recommended because uniform distribution of the nematode within these systems generally cannot be achieved.

C. Turfgrass

Commercial turfgrass grown in the United States is attacked by various insect pests that can be controlled by entomopathogenic nematodes. These include the immature stages of tropical sod webworm *(Herpetogramma phaeopteralis* Guenée), fall armyworm [*Spodoptera frugiperda* (J.E. Smith)], true armyworm [*Pseudaletia unipuncta* (Haworth)], and black cutworm [*Agrotis ipsilon* (Hufnagel)]. Other turfgrass pests controlled by nematodes are the billbugs (*Sphenophorus* spp.) and mole crickets *(Scapteriscus vicinus* Scudder and *S. borellli* Giglio-Tos). Although some of these pests occur at discrete times during the year, others can be year-round pests, especially in the southeastern United States. This discussion will be limited to stand establishment on golf courses; however, the principles can be applied in most turfgrass situations.

Turfgrass is a unique crop in that it is high value, yet economic thresholds for pest and disease control tactics are quite variable. On a golf course, for example, pest control decisions are often based on factors such as location of pest (fairway, tee, green, rough, etc.), time of year, size of budget, expectations of the membership, or even whether or not there is an upcoming event, such as a tournament. Thus, treatment patterns and thresholds can vary widely between golf courses, and even at the same course.

Stand establishment on a golf course can refer to the initial seeding of the course (called the "grow-in" phase), to areas on an established golf course that must be replaced due to damage from insects, diseases, etc., or to areas that are "overseeded," a process in which a cool-season turfgrass variety (e.g., ryegrass; *Lolium perenne* L.) is seeded over the top of an established turfgrass, which is dormant during the winter months. In all cases, the turf receives high levels of fertilizer (usually applied as a liquid through the irrigation system) and a tremendous amount of water to promote growth. Moreover, the seedling turfgrass is very susceptible to damage from spray equipment and foot traffic from maintenance personnel. Pest control under these conditions is difficult, at best. Chemical pesticides must be applied with ground rigs, which can damage the turf, and the intense irrigation tends to leach water-soluble pesticides before they can be effective.

Entomopathogenic nematodes have proven to be very effective under these conditions. Easily and accurately applied through the irrigation system, *S. carpocapsae* Steiner (the product Vector® TL), which targets caterpillars and billbugs, and *S. riobravis* (Vector® MC), which targets adult mole crickets, have been successfully used where traditional pesticides have failed. *S. scaptensci* Nguyen & Smart (Proactant Ss) is another nematode available for adult mole crickets, but is not recommended for application through the irrigation system. Their longevity, ease of application, environmental safety, and efficacy have made these products solid performers in the turfgrass industry. Although insect mortality is somewhat slower with nematodes, than with conventional insecticides, the efficacy of Vector® TL on caterpillars is excellent (Fig. 5) when used in a preventative treatment program. Vector® MC applications should target adult mole crickets, and significant reduction in cricket damage has been documented for up to 75 days after a single application (Fig. 6).

One example of using nematodes during turfgrass stand establish-

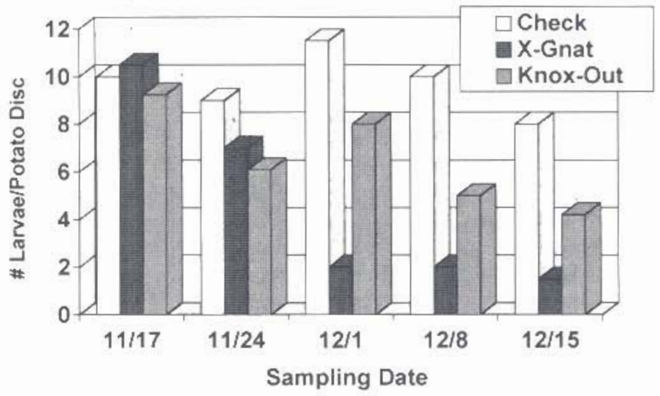

Fig. 4. Reduction in fungus gnat larvae on potted poinsettia following applications of X-Gnat *(Steinernema feltiae)* and KnoxOut (diazinon). Products applied at labeled rates on 11/17, 11/24, and 12/1 and evaluations made by counting fungus gnat larvae on potato discs placed on the soil surface. Trial conducted in Nov. 1994. (Unpublished data; M. Harris, Univ. of Georgia, Athens).

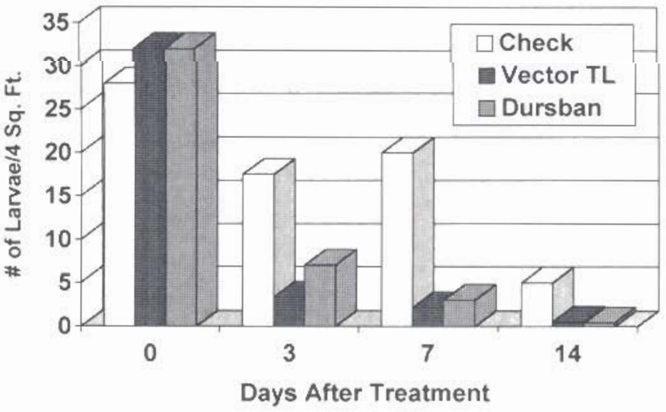

Fig. 5. Reduction in sod webworm larvae following a single application of Vector TL *(Steinernema carpocapsae)* and Dursban 4EC (chlorpyrifos). Products applied at labeled rates and evaluations made by visual count of target insects. Trial conducted in Sept. 1991. (Unpublished data; B. Drees, Texas A&M Univ., College Station).

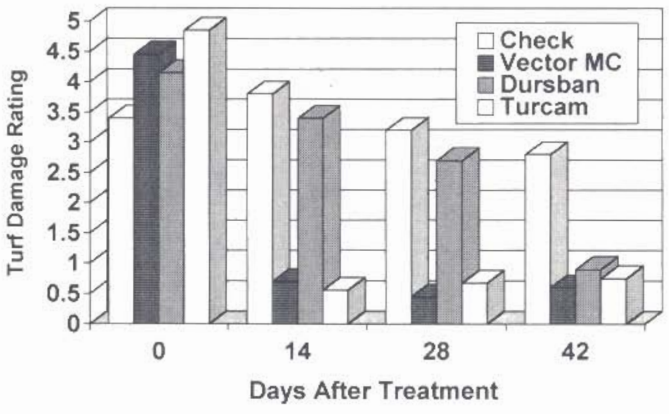

Fig. 6. Reduction in mole cricket damage to turfgrass following an application of Vector MC *(Steinernema riobravis)*, Dursban (chlorpyrifos) and Turcam (bendiocarb). Products applied at labeled rates and evaluations made by standard turfgrass damage ratings. Trial conducted in Apr. 1995. (Unpublished data; Leon Stacey, private consultant, St. Simons Island, Ga.).

ment involved a new golf course on St. Simons Island off the coast of Georgia. The seeding was under attack by fall armyworm, *S. frugiperda*. Repeated applications of the pesticide chlorpyrifos gave the superintendent little relief, mainly because it was being washed away by irrigation water. The effective life of chlorpyrifos under these conditions was only ≈24 h. Management was also concerned with continual pesticide applications because of the ecologically sensitive nature of the area. Vector® TL was applied over the entire golf course through the irrigation system and provided excellent and sustained control of armyworms for up to 6 weeks. Despite the slightly higher cost of the nematode product, this golf course has used Vector® TL on subsequent occasions, even though the turfgrass is no longer in the grow-in phase.

IV. CONCLUSIONS

Entomopathogenic nematodes have a variety of characteristics which make them desirable biopesticides—safety to vertebrates, no toxicity to nontarget arthropods, predictability, speed of kill, proven efficacy, and compatibility with conventional sprayers and irrigation systems. Many obstacles have been overcome in the effort to commercialize steinernematid and heterorhabditid nematodes, and current technology has allowed the successful introduction of nematode-based products in many markets, against a variety of soil-inhabiting insects.

Using entomopathogenic nematodes to control insect pests during the critical stand establishment phase of plant growth offers numerous advantages over traditional chemical pesticides. Often, during stand establishment, excess irrigation and damage from heavy spray equipment can limit the effectiveness of chemical pesticides. In addition, due to the sensitivity of the young plant during stand establishment, phytotoxic effects of some chemical pesticides make them undesirable. In many cases, entomopathogenic nematodes represent a viable alternative for conventional pest control during stand establishment.

As production and formulation technologies improve, the cost of nematode-based products will likely fall. Currently, in many markets, nematode-based products are competitive with agrochemicals. For example, BioVector 355 costs a citrus grower $20.00/grove acre application. Products will be enhanced by better formulations, making the nematodes easier to handle and store. Because nematodes can differ in their virulence and behavior, species and strain selection continue to be critical in the commercialization of nematodes. Finally, identifying and incorporating certain desirable traits (e.g., host-seeking ability, UV tolerance, cold tolerance) into nematodes through genetic manipulation may lead to more virulent strains that can better survive suboptimal environments.

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