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Effect of Application Timing of Oxamyl in Nonbearing Raspberry for *Pratylenchus penetrans* Management

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Abstract: In 2012, the Washington raspberry (*Rubus idaeus*) industry received a special local needs (SLN) 24(c) label to apply Vydate L® (active ingredient oxamyl) to nonbearing raspberry for the management of *Pratylenchus penetrans*. This is a new use pattern of this nematicide for raspberry growers; therefore, research was conducted to identify the optimum spring application timing of oxamyl for the suppression of *P. penetrans*. Three on-farm trials in each of 2012 and 2013 were established in Washington in newly planted raspberry trials on a range of varieties. Oxamyl was applied twice in April (2013 only), May, and June, and these treatments were compared to each other as well as a nontreated control. Population densities of *P. penetrans* were determined in the fall and spring postoxamyl applications for at least 1.5 years. Plant vigor was also evaluated in the trials. Combined results from 2012 and 2013 trials indicated that application timing in the spring was not critical. Oxamyl application reduced root *P. penetrans* population densities in all six trials. Reductions in *P. penetrans* population densities in roots of oxamyl-treated plants, regardless of application timing, ranged from 62% to 99% of densities in nontreated controls. Phytotoxicity to newly planted raspberry was never observed in any of the trials. A nonbearing application of oxamyl is an important addition to current control methods used to manage *P. penetrans* in raspberry in Washington.

Key words: Praytlenchus penetrans, postplant nematicide, root lesion nematode, *Rubus idaeus*, vydate.

Pratylenchus penetrans, the root lesion nematode, is a commonly found root parasite of raspberries (*Rubus idaeus*) in northwestern Washington (Gigot et al., 2013). If *P. penetrans* is not managed in raspberry, significant yield losses can occur (McElroy, 1977; Belair, 1991; Zasada et al., 2015). Pre- and postplant soil population densities of 100 *P. penetrans*/100 cm^3 and 140 to 550 *P. penetrans*/cm^3, respectively, have been proposed as thresholds for this nematode in raspberry (McElroy, 1992). *Pratylenchus penetrans* can complete several generations in a single growing season depending on soil temperature. Damage caused by *P. penetrans* generally includes a reduction in fine root abundance and the wounding of root tissue, which appears as necrotic lesions on the roots (McElroy, 1992).

Washington raspberry growers are accustomed to having a postplant nematicide available to help keep plant-parasitic nematodes in check. Before the cancellation of fenamiphos from the U.S. market in 2007 (USEPA, 2008), growers applied this product in the fall to bearing raspberry (1 year after planting) to control nematodes. Raspberry growers in Canada (many of whom are just across the border from their Washington counterparts) have a label for the use of oxamyl (Vydate L®; Dupont, Wilmington, DE). Current application recommendations for oxamyl in Canada include one application to be applied before October 31 at a rate of 9.35 liter/ha as a soil drench to bearing raspberry (Dupont, Registration No. 17995). In 2012, an SLN 24(c) label was issued for the application of oxamyl to nonbearing raspberry (within 3–5 mon after planting and at least 1 year before first harvest) in Washington (EPA Reg. No. 352-372). This is a departure from the way that Washington raspberry growers are familiar with applying postplant nematicides.

Oxamyl is a carbamate with contact and systemic activity; it has been shown to be an effective nematicide against many species of *Pratylenchus* in several production systems including potato (Hafez and Sundararaj, 2009), easter lily (Westerdahl et al., 2003), and mint (Rhoades, 1984). In raspberry, we demonstrated that a spring application of oxamyl to bearing raspberry plants reduced *P. penetrans* population densities for up to 2 years, but fall oxamyl applications were not effective (Walters et al., 2009). When trying to identify postplant nematicides alternatives for the Washington raspberry industry, oxamyl was superior to many of the other tested nematicides in suppressing *P. penetrans* (Zasada et al., 2010). Because of the proven efficacy of oxamyl against *P. penetrans* in raspberry, having this nematicide available to manage *P. penetrans* is an important tool for raspberry growers.

Although Washington raspberry growers can only apply oxamyl in the spring soon after planting, additional guidelines in best application methods for this nematicide are needed. The only application timing guidance the grower receives on the label is that soil temperatures need to be above 7°C. To help growers utilize this important postplant management option, the objective of this research was to investigate application timing of oxyaml to nonbearing raspberry for the suppression of *P. penetrans*.

Materials and Methods

Three trials each in 2012 and 2013 were established in grower fields near Lynden, WA (Table 1); each trial

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TABLE 1. Raspberry cultivar, location, soil type, and initial soil population densities of *Pratylenchus penetrans* in six trials included in oxamyl experiments in northwest Washington.

Trial[a]	Cultivar	Location (county)	Soil type	*P. penetrans*/ 100 g soil
1	Wakefield	Lynden	Tromp loam	0
2	Chemainus	Lynden	Kickerville silt loam	3 ± 1
3	Meeker	Lynden	Kickerville silt loam	1 ± 1
4	Meeker	Lynden	Tromp loam	0
5	Wakefield	Lynden	Kickerville silt loam	0
6	Wakefield	Everson	Mt. Vernon fine sandy loam	37 ± 6

Each trial was assessed as an individual experiment.

was comprised of a single cultivar. All of the fields had been fumigated with 1,3-dichloropropene plus chloropicrin the previous fall and were planted to raspberry in late March of each respective year. Raspberry 'Wakefield' was planted at tissue culture plugs while raspberry 'Meeker' and 'Chemainus' were planted as no. 1 canes. In each trial, an experimental unit (plot) was one-row wide (3 m), one-postlength long (9 m), and comprised 9 to 12 plants, with an identical number of plants in each plot within a trial. Before applying treatments, soil samples were collected to determine pretreatment population densities of *P. penetrans* (Table 1). From each plot, six soil cores (2.5 cm diameter × 20 cm deep) were collected, combined, and placed in a plastic bag. In the laboratory, the sample was mixed and a 50 g subsample was removed. Nematodes were extracted from the subsample using the Baermann funnel method (Ayoub, 1981). Nematodes were collected from funnels after 5 days and the number of *P. penetrans* determined using a stereo microscope at ×40. The *P. penetrans* populations were identified as *P. penetrans* based on morphological characteristics and the presence of males (Castillo and Volvas, 2007).

In all of the trials, treatments (application timings of oxamyl) were replicated four times and arranged in a randomized complete block design within a single row. In 2012, the following treatments were evaluated 1) oxamyl applied twice in May, 2) oxamyl applied twice in June, and 3) a nontreated control. In 2013, the same treatments were tested along with the inclusion of a fourth treatment, oxamyl applied twice in April. Applications were separated by 3 wk. At each application, oxamyl (Vydate L) at 2.2 kg/ha was applied with a CO_2-powered backpack sprayer in a volume of 430 liter/ha using a single nozzle boom (8006 nozzle; Teejet, Wheaton, IL) sprayed directly on the soil in a 1.2-m wide band centered on the row. Rates were calculated on the basis of the field acreage, but were concentrated in the band, as described previously (Walters et al., 2009). Each application was followed within 48 hours by rainfall or overhead water application of at least 1 cm. Nontreated plots received no nematicide and no additional overhead water application. Fertility, irrigation, and pest management practices were handled by the cooperating growers, and followed recommended practices for the region (Lower Mainland Horticulture Improvement Association, 2005).

Soil and root samples were collected from all trials and plots in the fall following treatment and then in the spring and fall for at least 1.5 years postapplication. From each plot, six soil cores (2.5 cm diameter × 20 cm deep) were collected, combined and placed in a plastic bag. For root collection, two 15 cm^3 cores were collected with a sharpened square-tipped trenching spade. The roots from a plot were collected and placed in the same bag as the soil sample. Nematodes were extracted from soil as described above. For extraction of *P. penetrans* from roots, the roots were washed free of soil and roots (<2 mm) were preferentially selected for extraction. Nematodes were extracted from roots using the intermittent mist method for 7 days (Ayoub, 1981).

Plots in all trials were evaluated for symptoms of phytotoxicity (stunting and leaf chlorosis) following treatment. In the 2012 trials, primocane number (the number of canes with basal diameter > 9 mm within 1 m of row, evaluated at three locations per plot) was determined 8 mon after planting. In trial 1 only, primocane height (height in centimeters of the three longest primocanes in each of three locations per plot) was also measured at the same time. Soil temperatures at application times were obtained from nearby AgWeatherNet stations (Lynden, Nooksack, and Lawrence, WA). AgWeatherNet (www.weather.wsu.edu) is maintained by Washington State University.

Data from each trial were analyzed separately. *Pratylenchus penetrans* data are presented as *P. penetrans*/100 g dry soil and *P. penetrans*/g dry root. Data were analyzed for effects of oxamyl treatment timing (treatment), sampling date (date), and treatment × date interactions using a repeated measure mixed linear model analysis of variance (ANOVA) with treatment and date as fixed effects and block as block as a random effect. *Pratylenchus penetrans* data were $\log_{10}(x+1)$ to correct for heteroscedasticity. Primocane height and number per meter of row were averaged for each plot. Averages were analyzed using one-way ANOVA with treatment as a fixed effect and block as a random effect. Treatment means in all analyses were separated using Tukey's honestly significant differences test ($P \leq 0.05$). All analyses were performed using JMP 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

In three of the trials, *P. penetrans* were undetectable in soil before establishing treatments (Table 1). *Pratylenchus penetrans* was detected in the remaining three trials with population densities ranging from 1 to 37 *P. penetrans*/100 g soil. A summary of the *P* values obtained from the repeated measure mixed linear model ANOVA from each trial location is presented in Table 2.

TABLE 2. Summary of *P* values from the repeated measure analysis of variance of the effect of oxamyl timing on population dynamics of *Pratylenchus penetrans* in soil and roots of raspberry.

Treatment	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	P. penetrans/100 g soil					
Date[a]	<0.001	<0.001	<0.001	<0.001	<0.001	0.0444
Treatment[b]	0.0263	<0.001	<0.001	0.4786	0.0943	0.0617
Date × time	0.7342	0.0123	0.8507	0.7486	0.8225	0.4139
	P. penetrans/g dry root					
Date	<0.001	<0.001	<0.001	<0.001	0.0048	0.8414
Treatment	0.1069	<0.001	<0.001	0.0017	0.0026	<0.001
Date × time	0.4871	0.0003	0.0489	0.6909	0.6647	0.0649

[a] Soil and root samples were collected to determine *P. penetrans* population densities up to 2.5 years after treatment in the spring and fall.

[b] Treatments included: nontreated control, and oxamyl applied in April (trials 4, 5, 6), May (all trials), and June (all trials). Each treatment was replicated four times in each trial.

In 2012, at the time of the first application of oxamyl in May and in June soil temperatures at 20 cm were 12.8°C to 14.2°C and 13.9°C to 15.1°C, respectively. Across the 2012 trials (1, 2, and 3) and sampling times, there were never differences in *P. penetrans* population densities in soil between the two oxamyl treatments (Table 3). This was also true for *P. penetrans* population densities in roots when a sampling date × time interaction was detected (Table 3). In trial 1, the main effect of sampling date was significant for both the soil and root data, while treatment was only significant for the soil data (Table 2). The May application timing of oxamyl resulted in 43% lower densities of *P. penetrans* in soil compared with the nontreated control (Table 3). In trial 2, there was an interaction between date and treatment for both the soil and root data (Table 2). Population densities of *P. penetrans* were lower in soil and roots, ranging from 75% to 98%, 6 mon and 1 year postapplication (Fall 2012 and Spring 2013, respectively), with treatment differences lost 1.5 years postapplication (Fall 2013; Table 3). In trial 3, both of the main effects in the model were significant for the soil data, with 80% lower population densities of *P. penetrans* in soil treated with oxamyl in May and June compared with the nontreated control (Table 3). The sampling date × treatment interaction was significant when the root data were considered. At the first two sampling dates, 6 mon and 1 year postapplication (Fall 2012 and Spring 2013, respectively), there were no difference in *P. penetrans* population densities in roots among the treatments. However, 1.5 and 2 years postapplication (Fall

TABLE 3. Population densities of *Pratylenchus penetrans* in soil and in roots of red raspberry cultivars after a postplant nonbearing applications of oxamyl in 2012.

Treatment[a]	Fall 2012	Spring 2013	Fall 2013	Spring 2014	Fall 2014
			P. penetrans/100 g soil		
Trial 1 (Wakefield)	B[b]	B	A	A	
Control (A)[b]	55[c]	44	450	175	nd[d]
May (B)	32	10	238	133	nd
June (AB)	18	24	218	203	nd
Trial 2 (Chemainus)					
Control	29 b[b]	53 b	213 a	nd	nd
May	1 c	2 c	54 b	nd	nd
June	1 c	5 c	85 ab	nd	nd
Trial 3 (Meeker)	B	B	B	B	A
Control (A)	28	25	63	50	450
May (B)	2	1	11	1	110
June (B)	0	3	6	3	74
			P. penetrans/g dry root		
Trial 1 (Wakefield)	BC	C	A	B	
Control	612	260	2,758	546	nd
May	175	320	1,412	259	nd
June	147	241	1,863	1,145	nd
Trial 2 (Chemainus)					
Control	103 ab	448 a	760 a	nd	nd
May	3 c	11 c	722 a	nd	nd
June	3 c	18 bc	1,003 a	nd	nd
Trial 3 (Meeker)					
Control	38 bcd	17 de	841 abc	498 a	539 a
May	2 de	2 de	5 de	33 cde	174 abc
June	2 de	1 e	10 de	22 cde	311 ab

[a] Location, raspberry variety, and month of application of oxamyl. Oxamyl was applied twice in each month at 2.2 kg oxamyl/ha in a volume of 430 liter/ha sprayed directly on the soil in a 1.2-m wide band centered on the row.

[b] Means separation using Tukey's honestly significant differences. Capital letters within a trial indicate a significant main effect difference in sampling data or treatment ($P < 0.05$; see Table 2). Values followed by different lowercase letters within a trial indicate a significant difference based upon the interaction of sampling date × treatment ($P < 0.05$; see Table 2).

[c] *Pratylenchus penetrans* population densities were $\log_{10}(x + 1)$ transformed for repeated measure analysis of variance and means separations; mean nontransformed data are presented.

[d] nd = not determined.

TABLE 4. Population densities of *Pratylenchus penetrans* in soil and roots of red raspberry varieties after postplant nonbearing applications of oxamyl in 2013.

Treatment[a]	Fall 2013	Spring 2014	Fall 2014
		P. penetrans/100 g soil	
Trial 4 (Meeker)	B[b]	B	A
Control	0	4	6
April	0	1	7
May	1	2	6
June	1	0	5
Trial 5 (Wakefield)	B	B	A
Control	3	2	21
April	6	4	75
May	0	0	5
June	1	1	54
Trial 6 (Wakefield)	B	AB	A
Control	63	83	92
April	35	99	154
May	30	32	75
June	22	14	95
		P. penetrans/g dry root	
Trial 4 (Meeker)	B	B	A
Control (A)[b]	16[c]	16	83
April (B)	2	4	16
May (B)	3	2	35
June (AB)	2	6	41
Trial 5 (Wakefield)	A	B	A
Control (AB)	152	20	105
April (A)	184	46	444
May (B)	52	7	49
June (A)	72	34	260
Trial 6 (Wakefield)			
Control (A)	916	692	529
April (A)	547	552	454
May (A)	891	341	331
June (B)	51	212	251

[a] Location, raspberry variety, and month of application of oxamyl. Oxamyl was applied twice in each month at 2.2 kg oxamyl/ha in a volume of 430 liter/ha sprayed directly on the soil in a 1.2-m wide band centered on the row.

[b] Means separation using Tukey's honestly significant differences. Capital letters within a trial indicate a significant main effect difference in sampling data or treatment ($P < 0.05$; see Table 2). Values followed by different lowercase letters within a trial indicate a significant difference based upon the interaction of sampling date × treatment ($P < 0.05$; see Table 2).

[c] *Pratylenchus penetrans* population densities were $\log_{10}(x+1)$ transformed for repeated measure analysis of variance and means separations; mean non-transformed data are presented.

2013 and Spring 2014, respectively) population densities of *P. penetrans* were significantly lower, ranging from 93% to 99%, in roots of plants grown in plots treated with oxamyl in May or June compared with the nontreated control. This effect was lost at the last sampling date, 2.5 years postapplication (Fall 2014). No phytotoxicity (leaf chlorosis or stunting) to raspberry was observed at any point in the 2012 trials. Primocane height was measured in trial 1; primocanes in nontreated, May-treated, and June-treated plots averaged 224, 230, and 229 cm, respectively. The primocane heights were not significantly different among treatments at trial 1 ($P = 0.7836$). Primocane number per meter of row in 2012 ranged from 3.8 to 4.6, 8.2 to 9.1, and 5.7 to 8.0 in trials 1, 2, and 3, respectively. There were no differences in primocane number among the treatments in any trial ($P > 0.2324$).

In 2013 (trials 4, 5, and 6), oxamyl was applied twice beginning in April, May, or June. At the time of the first application of these treatments soil temperatures at 20 cm were 10.0°C to 11.3°C, 10.8°C to 13.2°C, and 14.9°C to 17.1°C in April, May, and June, respectively. There was never a difference in population densities of *P. penetrans* in soil in any of the 2013 trials due to oxamyl treatment compared with the nontreated control (Tables 2,4). The main effect of sampling date was significant with generally higher population densities of *P. penetrans* found in soil later in the life of the raspberry plantings. When the root data were considered, the effect of oxamyl treatment was significant in all of the trials while sampling data were only significant in trials 4 and 5 (Table 2). In both trials 4 and 5, population densities of *P. penetrans* in roots were 69% and 62% lower, respectively, in plants treated with oxamyl in May compared with the nontreated control (Table 4). In trial 4, the April application of oxamly also resulted in 88% fewer *P. penetrans* in roots compared with the nontreated control. In trial 6, population densities of *P. penetrans* in roots were 75% lower in plots treated with oxamyl in June compared to all other treatments (Table 4). No phytotoxicity to raspberry was observed at any point in the 2013 trials.

DISCUSSION

Application of postplant nematicides in the spring is a departure from how raspberry growers have long used these products. Fall application of fenamiphos to raspberry was recommended for years (McElroy, 1992), and currently the Canadian label for Vydate L only allows for applications in the fall. Results from our study, and from previous studies, demonstrate that spring applications of oxamyl are effective in suppressing *P. penetrans*. Pinkerton et al. (1983) showed that spring applications of oxamyl to peppermint (*Mentha* × *piperita*) suppressed *P. penetrans*, but fall applications were ineffective. A similar observation was made in raspberry (Walters et al., 2009). The goal of this study was to evaluate the efficacy of oxamyl applied at different times in the spring to nonbearing raspberry. Combined results from 2012 and 2013 trials indicated that application timing in April, May, or June is not critical. Application later than June would not permit harvest of raspberry fruit the following year, due to the limitations of the 24(c) label for Vydate L application to raspberry in Washington.

Oxamyl has a low persistence in soil with reported half-lives of 4 to 20 days (Holt and Leitch, 1978). There are several abiotic factors that will influence oxamyl degradation in soil. First and foremost is soil temperature. In a model of oxamyl degradation in 10 soils, soil temperature accounted for 79% of the variation in the model, a much greater impact on degradation than rainfall (Haydock et al., 2012). Lower soil temperatures retard the degradation of oxamyl, maintaining concentrations of the product that are lethal to *P. penetrans*. In northern Washington average soil temperatures (2009–2014) in April, May, and June are 10.7°C, 14.6°C, and 17.3°C, respectively. Based on soil temperatures it would be expected that oxamyl will persist the longest when applied in April in northern Washington. However, in three different soil types, the rate of oxamyl degradation was greater between soil temperatures of 5°C to 10°C than between 10°C and 15°C (Barmilow et al., 1980). Therefore, whereas oxamyl concentrations in soil were likely higher in April than May or June, potentially this difference was not as large due soil temperatures being >10°C at all application dates. Soil type is another factor that can impact oxamyl degradation and absorption (Barmilow et al., 1980; Haydock et al., 2012). Our trials encompassed several different soil types (loam, sandy loam, silt loam) and we did not observe any noticeable differences in oxamyl efficacy across these soil type. At all sites *P. penetrans* suppression was observed on at least one sampling date after the application of oxamyl.

It is extremely important to move oxamyl into the soil after a surface application to minimize degradation. This is readily achieved with irrigation or rainfall because oxamyl is highly soluble in water (Holt and Leitch, 1978). The 24(c) label for Vydate L states that rainfall should be imminent or overhead irrigation should be used to incorporate oxamyl as soon after application as possible. However, the majority of the raspberry acreage in northern Washington is drip irrigated, making the even application of water to a 1.2-m area treated with oxamyl difficult. The average amount of rainfall in northern Washington (2009–2014) in April, May, and June is 7.8, 7.5, and 3.7 cm. Based on these historical data, it is more likely that growers will be able to time a banded application of oxamyl with imminent rainfall in April or May.

Oxamyl is an effective postplant nematicide, but it does not eliminate all nematodes from an application area. In controlled experiments, oxamyl reduced population densities of *P. penetrans* in raspberry roots by 88% compared with the nontreated control (Zasada et al., 2010). In field trials, two banded applications of oxamyl in spring reduced population densities of *P. penetrans* in roots of raspberry 'Willamette' by 94% compared with the nontreated control (Walters et al., 2009). In the trials presented here, when treatment effects were significant population densities of *P. penetrans* were reduced >90% in soil and/or roots.

Raspberry varieties are similar in their ability to support *P. penetrans* and in the extent of damage caused by *P. penetrans* (Zasada et al., 2015). Therefore, we expected initial *P. penetrans* population densities to be a more important factor in our experiments than variety. In all of the trials except one (trial 6), there were undetectable levels of *P. penetrans* in soil before establishment of treatments. However, in all trials 6 mon after pretreatment samples were collected *P. penetrans* was found in both soil and root samples. In the nontreated controls in trials 1, 2, and 3, these *P. penetrans* population densities in soil would be considered moderate to high (>140 *P. penetrans*/100 cm^3 soil; McElroy, 1992). Pretreatment samples were collected to a depth of 15 cm, a depth which would be expected to be well treated with a broadcast, shank application of the soil fumigant 1,3-dichloropropene plus chloropicrin injected to a depth of 40 to 45 cm. *Pratylenchus penetrans* are migratory ectoparasites and it is possible that *P. penetrans* are surviving below this depth serving as a reservoir to infect newly planted raspberry. In a sandy loam soil, *Pratylenchus brachyurus* was found down to 105 cm with population densities greatest at 45 to 75 cm (Brodie, 1976). Across soil types in wheat fields in Australia, *P. neglectus* and *P. thornei* were found down to a depth of 90 cm; however, the majority of the population was found in the upper 0 to 10 cm (Taylor and Evens, 1998). In raspberry, population densities of *P. penetrans* were found to be greater at 5 to 10 cm (Forge et al., 1998); however, densities of *P. penetrans* were only considered down to 30 cm. Another scenario is that *P. penetrans* may have survived fumigation within soil clumps or root pieces or within upper parts of the soil profile, which experience lower fumigant levels than deeper in the soil profile (Gao et al., 2008). Knowledge of where *P. penetrans* populations are residing in the raspberry production system after fumigation is an important factor to understand to maximize the efficacy of oxamyl. Currently, this is not well understood in this production system.

Phytotoxicity of nonbearing applications of oxamyl in the spring was not observed on newly planted (1- to 3-mon old) raspberry 'Wakefield', 'Meeker', or 'Chemainus'. Raspberry 'Meeker' and 'Wakefield' comprise >80% of the planted acreage in northwest Washington (P. Moore, pers. comm.). These results do not mean that other raspberry varieties might not react differently to applications of oxamyl. Spring applications of oxamyl reduced yield of bearing 'Nootka' but not of 'Willamette'; no yield reduction in either variety was noted with fall applications of oxamyl to bearing raspberry in the same study (Walters et al., 2009).

A nonbearing application of oxamyl is an important addition to current control methods used to manage *P. penetrans* in raspberry in Washington. Raspberry growers have flexibility in application timing in the spring after planting as long as applications occur 12 mon or more prior to harvest. Based upon historical soil temperatures and rainfall in the region, applications beginning in May have the potential to be more efficacious because soil temperatures are higher than in April and rainfall is more abundant than in June. The use of a nonbearing application of oxamyl will have longer term benefits in fields with lower *P. penetrans* pressure.

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