

Biological Control of *Rhizoctonia solani* with a Soil-Inhabiting Basidiomycete

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ABSTRACT

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An isolate of a soil-inhabiting unidentified *Corticium* sp. which was parasitic to *Rhizoctonia solani* in culture, also was an effective biological control agent of this pathogen of sugar beets in greenhouse studies. Seed coated with mycelia and sclerotia of *Corticium* and planted in nonsterilized soil artificially infested with *R. solani*, produced significantly greater seedling stands than did nontreated seeds in the same soil. In a field study, *Corticium*-coated seeds of sugar beet planted in soil naturally infested with *R. solani* produced seedling stands more than 100% greater than untreated seeds. Nontreated sugar beet seed produced significantly greater seedling stands in soil simultaneously infested with *R. solani* and *Corticium* than in

soil with *R. solani* only, if planted 2–15 wk after infestation. In greenhouse studies, high percentages of seedling establishment and low soil propagule densities of *R. solani* were correlated with high soil densities of *Corticium*. In the greenhouse and field, more consistent establishment of high soil populations of *Corticium* occurred if dry sugar beet pulp was mixed with *Corticium* prior to soil incorporation than if *Corticium* was incorporated alone. *Corticium* exhibits morphological and physiological stability in culture and longevity (>3 yr) when stored as an air-dry preparation of mycelia and sclerotia under nonsterile conditions.

Additional key words: hyperparasitism, damping-off, crown rot, soil augmentation, *Beta vulgaris*, *Laetisaria* sp.

While several biological control systems have been described for soilborne plant pathogens, few have been successful in field experiments (1,2,11) and of those, only one is economically feasible (1). Several biological control systems have been reported for *Rhizoctonia solani* Kuhn, but none have proven effective in the field (2,3,5,6).

A basidiomycetous fungus obtained from sugar beet residue in soil of western Nebraska several years ago (Boosalis, unpublished) parasitized *R. solani* in culture and thus was selected for evaluation as a biological control of diseases caused by *R. solani*. A preliminary report (9) described studies evaluating this basidiomycete for biological control of *R. solani* on soybeans (*Glycine max* [L.] Merr.), dry edible beans (*Phaseolus vulgaris* L. 'Great Northern'), and sugar beets (*Beta vulgaris* L.). Subsequent research by others demonstrated additional biological control potential of this organism against *R. solani* on cucumbers (8) and *Pythium ultimum* Trow on table beets (7).

The same basidiomycete isolate used in all of these studies is temporarily classified as a *Corticium* sp. (7) but recently, this and similar isolates have been included in a newly-described species of *Laetisaria* (4).

This report describes our investigation of one isolate of the *Corticium* sp. as a biological control agent of *R. solani*-caused diseases (damping-off and crown rot) of sugar beets. The various production methods and carrier systems investigated for increase of *Corticium* in culture and in field soil are reported and discussed.

MATERIALS AND METHODS

Production of *Corticium*. Inoculum of *Corticium* was increased in still culture of potato dextrose broth (PDB) either on 50 ml in 250-ml Erlenmeyer flasks or on 15 ml in glass petri dishes (9 cm in diameter). Each liter of PDB contained decoction from 300 g of potato and also contained 20 g dextrose. After 9–14 days of

incubation at 24–26 C, the mycelial-sclerotial mixture was collected, air-dried, and ground through a 0.85 mm pore size (20-mesh) screen in a Wiley mill. This "coarse" preparation was used in all experiments except where specifically stated that a "fine" preparation was used. The fine preparation was prepared by grinding the air-dry material in a blender at high speed. All *Corticium* preparations were stored air-dry prior to use in nonsterile glass or plastic containers at 24–26 C. Cornmeal, cornmeal and sand, perlite impregnated with molasses (1) or PDB, diatomaceous earth granules impregnated with molasses or PDB, ground sugar beet pulp, and PDB were evaluated as cultural substrates for increasing inoculum of *Corticium*. PDB was the best medium for growing *Corticium*.

In the latter part of the study, *Corticium* was increased in fermentation culture in PDB. After 5–7 days of incubation at 25 C, *Corticium* growth was collected and processed as described above. Aeration and agitation rates were varied, but no optimal rates were determined; all produced similar growth patterns and amounts of mycelium. *Corticium* inoculum produced by fermentation was similar in viability and activity to that prepared in still cultures of PDB, but it had a higher sclerotia:mycelium ratio.

Production of *R. solani* inoculum and infestation of soil. Inoculum of isolates of *R. solani* for soil infestation was increased aseptically on a cornmeal-vermiculite medium in 250-ml Erlenmeyer flasks. The medium contained 5 g cornmeal, 5 g vermiculite, and 25 ml of distilled water. A plug of inoculum (5 mm diameter) from the periphery of a 5-day-old culture of *R. solani* grown on potato dextrose agar (PDA) in petri plates was transferred into the Erlenmeyer flask medium and incubated 1–2 wk at 24–26 C. The cultures were shaken periodically to produce uniform growth of *R. solani*.

Approximately one flask (10 g dry weight) of one or more isolates of *R. solani* was incorporated per 2,000–4,000 cm³ of soil. To minimize inoculum variability between replications and between experiments, inoculum of several flasks were pooled and the approximate wet-weight equivalent for a single flask was calculated. Typically, large volumes of soil were infested with the

appropriate amount of pooled inoculum to achieve a uniform density of *R. solani* prior to subdivision into smaller aliquots for specific treatments. Propagule densities of *R. solani* in the artificially infested soils were always initially greater than those in the field. The soil used in all greenhouse experiments was a nonsterilized sandy loam soil collected from sugar beet fields in western Nebraska.

Coating of seeds with inoculum of *Corticium*. Sugar beet seeds were coated by dipping them into a 1% w/v methyl cellulose solution and rolling them in a coarse or fine *Corticium* inoculum preparation. The coated seeds were dried for at least 12 hr prior to use.

Analysis of densities of *R. solani* and *Corticium* in soil. A modification of the sugar beet seed colonization method (10) was used as an indirect method to determine densities of *R. solani* and *Corticium* in soils of both greenhouse and field experiments. This method was more useful for determining the relative differences in population density of the two fungi than for quantifying the actual propagule density of either fungus.

In greenhouse experiments, equal amounts of soil (100 cm³ or more) were collected from each replication, pooled by treatments, mixed, and, for each treatment, a single 200-cm³ soil sample was removed for analysis. In field experiments, equal amounts of soil (100 cm³ or more) were collected from separate areas of the row for each replication, pooled by treatment replication, mixed, and a 200-cm³ sample was removed for analysis.

Fifty autoclaved, dry, sugar beet seeds were thoroughly mixed into a 200-cm³ soil sample in plastic bags and the soil was adjusted to 50% water holding capacity (WHC). If possible, wet soil was allowed to dry to near 50% WHC. The bags with soil and sugar beet seeds were loosely rolled into cylinder shapes to maintain moisture, but permit gaseous exchange. The bags were incubated at 24–26 C for 2–3 days after which the sugar beet seeds were recovered by wet-sieving the soil over a 1.7-mm pore-size (10-mesh) screen. High-pressure water streams removed nearly all adhering soil from the seeds. After allowing the seeds to air-dry for 30 min on filter paper, they were placed on water agar (WA). After incubation for 24 hr at 24–26 C, colonies growing onto WA from the approximately 40 retrieved seeds were examined microscopically and colonies of *R. solani* and *Corticium* were recorded as the percentage of seeds colonized. To identify suspect colonies of *R. solani* and *Corticium*, mycelial transfers were made to PDA and comparisons of colony and mycelial morphology were made to known characteristics of the *Corticium* isolate and the various isolates of *R. solani* used to infest the soil.

Seed coating experiments in the greenhouse. Sugar beet seed (cultivar Mono HyD2, Great Western Sugar Co. Research Center, Longmont, CO 80501) was coated with a coarse or fine preparation of *Corticium*, methyl cellulose only, pentachloronitrobenzene (PCNB), 0.48 mg active ingredient (a.i.) per gram of seed (LT-2, Olin Corp., Agricultural Div., Little Rock, AK 72203), or seed was

not treated. Soil was left noninfested or infested with inoculum of *R. solani* isolate RS 18 which was pathogenic to and originated from sugar beets. Four replications of each treatment were done in 500-cm³ pots and 10 treated, coated, or nontreated seeds were planted in the soil of each pot immediately after infestation with *R. solani*.

“Average percentage plant stand” (seedling stand based on seedlings established/seeds planted) was typically determined within 2–3 wk from planting when seedling emergence and post-emergent damping-off had ceased.

Field experiments with coated seed. Seeds of sugar beets were left nontreated or coated with viable or autoclaved preparations of *Corticium* and planted in areas of a field in western Nebraska having a high incidence of crown rot of sugar beets. A total of 1,000 seeds was planted for each treatment in unequal numbers and lengths of row. Total plant stands for the entire row length of each treatment were recorded periodically for 2 wk following planting.

***Corticium* used as a soil augmentation in greenhouse studies.** Soil was augmented with *Corticium*, 0.2% w/v, PCNB, 0.1% w/v formulation, (Terraclor 10G [10% granular formulation], Olin Corp.), or left nontreated. Soil of each treatment was divided into two aliquots, one was left noninfested and the other was infested with inoculum of *R. solani* from isolates pathogenic to sugar beets, soybeans, and dry edible beans. Tests were conducted in 2,000-cm³ pots with eight replications of each treatment in infested and noninfested soil. Thirty nontreated sugar beet seeds (Mono HyD2) were planted in all soils immediately after infestation with *R. solani* was completed. Average percentage plant stands were determined as previously described but within a 2- to 4-wk period, after which the remaining plants were removed, and similar successive plantings were made to determine how protection against *R. solani* varied with time after the initial treatment. Average percent plant stand was determined for each planting of each treatment and densities of *R. solani* and *Corticium* in soil were evaluated at experiment termination (20 wk).

Sugar beet pulp as an organic carrier for *Corticium* in greenhouse studies. Sugar beet pulp pellets (Great Western Sugar Co., Scottsbluff, NE 69361) were ground to a particle size similar to the coarse *Corticium* preparations (≤ 0.85 mm). The nonsterilized, dry, ground sugar beet pulp (SBP) was mixed with dry, coarse, *Corticium* preparation just prior to incorporation of the augmentation (*Corticium*:SBP) into soil. The level of *Corticium*:SBP augmentation of the soil in the greenhouse was usually adjusted to incorporate *Corticium* at 0.2% w/v to facilitate comparisons with experiments described in the previous section.

In greenhouse experiments, soil augmentation treatments included *Corticium*:SBP (1:8.5, 1.9%, w/v), *Corticium* only (0.2% w/v), SBP only (1.7% w/v), and nontreated soil. Soil of all treatments was watered after augmentation. Incubation for 2 days at 26 C was allowed to permit colonization of SBP in soils by *Corticium*. After the incubation period, all soils were infested with inoculum of two isolates that induce crown rot of sugar beets (B-1 and B-2) and one isolate (RS 69) from soybeans also pathogenic to sugar beets and known to have longevity in soils in the greenhouse. All procedures were the same as those described in the previous section except that propagule densities of *R. solani* and *Corticium* were evaluated 5 wk after recording average percentage plant stands of the fifth (final) planting.

Diatomaceous earth granules as a carrier for *Corticium* in field studies. Abbott Laboratories, Chicago, IL 60064, produced sufficiently large amounts of a *Corticium* preparation to augment larger areas of field soil than was possible if our laboratory methods were used to increase *Corticium*. However, in this *Corticium* preparation (grown on diatomaceous earth granules) germination was delayed and there was less vigorous growth of mycelia on WA when compared with *Corticium* inoculum prepared in our laboratory. The *Corticium* preparation grown on diatomaceous earth granules was evaluated as a soil-incorporated, *R. solani*-suppressive agent in soil of a sugar beet field in western Nebraska. Just prior to the planting of beets in April, the *Corticium* preparation (0.2%, w/v) was incorporated 5 cm deep in a 10-cm band of the row. Each replication was a single row 6 m long. The

TABLE 1. Comparison between *Corticium* and pentachloronitrobenzene (PCNB) as a sugar beet seed protectant against *Rhizoctonia solani* in soil

Seed treatment ^a	Average percentage plant stand ^b	
	Noninfested soil	<i>R. solani</i> -infested soil
Nontreated	64 ab	5 f
Methyl cellulose	47 cd	2 f
<i>Corticium</i> inoculum, coarse	67 a	55 bc
<i>Corticium</i> inoculum, fine	62 ab	24 e
PCNB	44 d	31 e

^aSeeds were dipped in a 1% methyl cellulose solution, removed and allowed to dry or rolled in one of the two *Corticium* inoculum preparations and allowed to dry. Seeds treated with PCNB received 0.48 mg a.i./g of seed. Each of four replications for each treatment contained 10 seeds per 500 cm³ of soil.

^bSeedling stand, based on the number of seedlings established per number of seeds planted, was determined within 2–3 wk from planting and after seedling emergence and postemergent damping-off had ceased. Values followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

experiment utilized a completely randomized block design with 10 replications of each treatment. Nontreated sugar beet seed (Mono HyD2) was planted in all augmented and nonaugmented soils. Stand counts were taken throughout the season and crown rot percentages were recorded periodically in all treatments after the disease first appeared.

Sugar beet pulp as an organic carrier for *Corticium* in field studies. A field experiment was conducted to determine the effect of SBP on the establishment of *Corticium* in field soils. *Corticium* inoculum was produced in fermentation culture and it was similar to previous coarse preparations. *Corticium*:SBP augmentations (ratio, % w/v) of (1:6, 1.4), (1:60, 1.22), (1:20, 0.42), and *Corticium* at 0.2 and 0.02% w/v and SBP at 0.4 and 1.2% w/v were incorporated 5 cm deep in 10-cm-wide bands or soil was nonaugmented. Each replication was a single row of sugar beet 3 m long. The experiment utilized a completely randomized block design with four replications and was conducted in two western Nebraska sugar beet fields. After a 4-wk incubation period, soil propagule densities of *R. solani* and *Corticium* were analyzed.

RESULTS

***Corticium* used as a seed coating.** In greenhouse studies, sugar beet seeds coated with fine and coarse *Corticium* inoculum produced significantly greater average percent plant stands after 2–3 wk in *R. solani*-infested soil than did nontreated seed (Table 1) and the stands were comparable to those achieved with PCNB-treated seeds. Although not apparent in every experiment, *Corticium*-coated seeds usually produced greater stands in noninfested soil than did nontreated seed. Seeds of sugar beet coated with *Corticium* produced 105% greater plant stands (not statistically analyzed) than did nontreated seed or seed coated with autoclaved *Corticium* when planted in a western Nebraska sugar beet field with a high incidence of crown rot.

Evaluation of *Corticium* as a soil augmentation to suppress *R. solani*. In greenhouse studies of nontreated sugar beet seed, soils with dry *Corticium* incorporated simultaneously with *R. solani* usually did not have average percent plant stands greater than those in soil with *R. solani* only, until plantings were made 2–20 wk after infestation (Table 2). The greater average percent plant stands in these *Corticium*-augmented soils were comparable to those in *R. solani*-infested soil augmented with PCNB. In several experiments in soil without *R. solani* infestation the average percent plant stands in *Corticium*-augmented soil were significantly greater than those in nonaugmented or PCNB-treated soil (Table 2). The highest average percent plant stand occurred in soil with the highest *Corticium* density and one of the lowest *R. solani* densities (Table 2). The lowest average percent plant stand occurred in soil with the

highest *R. solani* density and no detectable *Corticium*. Neither *Corticium* nor *R. solani* were detectable in the PCNB-treated soils (Table 2) when analyzed by our method.

Carrier systems for *Corticium* used as a soil augmentation. Field soil (in situ) augmented with the diatomaceous earth preparation of *Corticium* at planting time (April) had significantly higher plant stands than nonaugmented soil at mid-season (August), but there were no significant differences at harvest (October). A large amount of crown rot developed in these plots (24–36%) by October, but there were no significant differences between *Corticium*-augmented and nonaugmented soils.

Preliminary studies indicated that soils augmented with various ratios and rates of *Corticium*:SBP typically had higher *Corticium* densities than did soils augmented with similar rates of each material alone. Two days after incorporation, *Corticium* was easily isolated from SBP particles recovered from soil augmented with *Corticium*:SBP but not from soil augmented with SBP only. In greenhouse studies, the average percentage plant stand in a fifth planting (15 wk after augmentation) was significantly higher in *R. solani*-infested soil augmented with *Corticium*:SBP (1:8.5, 1.9% w/v) than in soil augmented with either material alone or nonaugmented (Table 3). The highest *Corticium* and lowest *R. solani* densities occurred in soil augmented with *Corticium*:SBP (Table 3).

All soil augmented in the field with any ratio and rate of *Corticium*:SBP had a significantly higher *Corticium* density 4 wk after incorporation than did soil augmented with any rate of *Corticium* or SBP alone (Table 4). The soil augmented with the highest rate of both *Corticium* (0.2% w/v) and SBP (1.2% w/v) had the highest *Corticium* density (Table 4). During density analyses we noted that mycelial growth of *Corticium* on WA from sugar beet seeds incubated in this latter soil was more vigorous and occurred more rapidly than growth from seeds incubated in the soils augmented with other ratios and rates of *Corticium*:SBP or *Corticium* alone. The apparent differences in *Corticium* densities between locations I and II (Table 4) may not be as great as indicated since soil samples at location II were analyzed at near field capacity moisture. At the time of density analysis, densities of *R. solani* in all soils at both locations had declined to levels almost nondetectable by our method.

DISCUSSION

Greenhouse and field results indicate that our isolate of *Corticium* may be useful as a biological control agent of *R. solani*. We demonstrated that soils in the greenhouse with high propagule densities of *Corticium* had low densities of *R. solani* and significantly greater average percentage plant stands of sugar beets than soils with low densities of *Corticium* and high densities of *R.*

TABLE 2. Efficacy of *Corticium* as a soil augmentation to protect nontreated sugar beet seed against *Rhizoctonia solani* in artificially infested field soil in the greenhouse^a

Soil augmentation	<i>R. solani</i> infestation (+ or -)	Average percentage plant stand in fifth planting (20 wk) ^b	Density in soil after fifth planting (20 wk) (% beet seed colonization)	
			<i>Corticium</i>	<i>R. solani</i>
None	-	33 a	0	29
<i>Corticium</i>	-	50 b	50	10
PCNB	-	30 a	0	0
None	+	37 a	0	34
<i>Corticium</i>	+	50 b	82	8
PCNB	+	53 b	0	0

^a Soil augmentations (*Corticium* at 0.2% w/v or pentachloronitrobenzene at 0.1% w/v) and *R. solani* inoculum were added to soil simultaneously.

^b Each of eight replications for each treatment contained 30 seeds per 2,000 cm³ of soil. Seedling stand, based on the number of seedlings established per number of seeds planted, was determined within 3–4 wk from planting in five successive plantings. Values followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 3. Efficacy of sugar beet pulp as a carrier for *Corticium* utilized as a soil augmentation to protect nontreated sugar beet seed against *Rhizoctonia solani* in artificially-infested sugar beet field soil in the greenhouse^a

Soil augmentation			Average percentage plant stand in fifth planting (15 wk) ^b	Density in soil after fifth planting (20 wk) (% beet seed colonization)	
<i>Corticium</i> (% w/v)	Sugar beet pulp (ratio)	(% w/v)		<i>Corticium</i>	<i>R. solani</i>
0	...	0	9 a	0	76
0.2	...	0	13 a	29	55
0	...	1.7	2 a	0	87
0.2	(1:8.5)	1.7	67 b	79	0

^a Dry sugar beet pulp and the *Corticium* preparation (both ≤ 0.85 mm particle size) were mixed just prior to incorporation into soil. Two days after incorporation all soils were infested with *R. solani*.

^b Each of eight replications for each treatment contained 30 seeds per 2,000 cm³ of soil. Seedling stand, based on the number of seedlings established per number of seeds planted, was determined within 3 wk from planting in five successive plantings. Values followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 4. The effect of sugar beet pulp on the establishment of *Corticium* in sugar beet field soil at two locations in western Nebraska^a

Soil augmentation			<i>Corticium</i> density 4 wk after incorporation	
<i>Corticium</i>	Sugar beet pulp		(average % beet seed colonization) ^b	
(% w/v)	(ratio)	(% w/v)	Location I	Location II
0	...	0	0 a	0 a
0.2	...	0	18 b	11 b
0.02	...	0	4 a	2 a
0	...	1.2	0 a	0 a
0	...	0.4	1 a	0 a
0.2	1:6	1.2	65 d	30 d
0.02	1:60	1.2	49 c	39 d
0.02	1:20	0.4	50 c	20 c

^aDry sugar beet pulp and the *Corticium* preparation (both ≤ 0.85 mm particle size) were mixed just prior to incorporation into soil. Augmentations were incorporated 5 cm deep in a 10-cm band of the row. Each of four replications was 3 m long.

^bFor each replication, 50 sterile sugar beet seeds were placed in a 200-cm³ soil sample adjusted to approximately 50% water holding capacity (except soil from location II which was near field capacity) and incubated at 24–26 C for 2 days. Seeds were retrieved on a screen, incubated on water agar for 24 hr at 24–26 C, and the percentage of seeds colonized by *Corticium* was recorded. Values in the same column followed by the same letter are not significantly different, $P=0.05$, according to Duncan's multiple range test.

solani (Tables 2 and 3). When similar soils were augmented with identical rates of dry, coarse *Corticium* preparations (mycelia and sclerotia), *Corticium* did not uniformly become established in high densities (Tables 2 and 3). Greenhouse and field studies determined that sugar beet pulp (SBP), used as an organic carrier with coarse *Corticium* preparations, significantly and consistently increased propagule densities of *Corticium* compared to those in soils augmented with *Corticium* or SBP alone (Tables 3 and 4).

The mechanism responsible for biological control of *R. solani* by *Corticium* was not identified. It is possible that the parasitization of *R. solani* by *Corticium* in culture may have little or no role in biological control. Control mechanisms other than parasitism could be involved because Hoch and Abawi (7) were able to demonstrate that *Corticium* is nonpathogenic to *P. ultimum* even though it is an effective biological control agent of this organism. In our studies, the rapid colonization of certain substrates (sugarbeet seed coats and SBP) in soil by *Corticium* suggest competitive inhibition as a possible control mechanism to be considered along with parasitism and the antiobiosis suggested by Hoch and Abawi (7).

Our greenhouse studies demonstrated that high propagule densities of *Corticium* established in soils were maintained for several weeks and substantially reduced the initially high inoculum density of *R. solani* (Tables 2 and 3). Lewis and Papavizas presented similar data (8). Establishment of high propagule densities of *Corticium* in soil early in the growing season might effectively retard increase of *R. solani* and reduce disease incidence in a manner similar to that described by Wells et al (11) for *Trichoderma harzianum* against *Sclerotium rolfsii*.

If useful as a biological control of diseases caused by *R. solani* or other organisms, *Corticium* has many characteristics that make it attractive for commercial development. *Corticium* is adaptable to

fermentation culture and has a rapid growth rate over a wide temperature range on numerous growth media. Our isolate of *Corticium* has demonstrated morphological and physiological stability (eg, parasitism, biological activity) and air-dry preparations of *Corticium* have longevity (>3 yr) at room temperature (24–26 C). The dry *Corticium* preparations can be mixed with a nonsterile, dry, organic carrier (SBP) at any time prior to incorporation into soil thus obviating carrier sterilization or previous growth of *Corticium* on carrier materials. *Corticium* has not demonstrated parasitism to seed or seedlings of any crop (7,9), is active in soil, and may be useful as a seed coating material.

From many different Nebraska soils, we obtained fungal isolates similar to our initial isolate of *Corticium*. In culture, these fungal isolates were indistinguishable morphologically from our initial isolate of *Corticium* and of those tested, all were parasitic to *R. solani*. Morphologically similar isolates have been obtained from soils of Maryland and Ohio (4). *Corticium* may be a ubiquitous soil-inhabiting organism and is apparently an undescribed species (4).

The recent interest in *Corticium* as a biological control agent and previously undescribed component of the soil microflora provides exciting opportunities for future biological control research with this organism. Increased knowledge of its distribution, and ecology in soils will help to more adequately evaluate and exploit it as a biological control agent against *R. solani* and other organisms. Other strains of this organism possibly exist which are superior biological control agents to the single isolate demonstrating such potential in this and other studies.

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