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Impact of *Pythium* Infection on Vegetable Production and Its Management

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Abstract

Pythium species are one of the most devastating soil-borne pathogenic of vegetable crops in the world, which has been known to cause seed rot, damping-off, root rot and collar rot at all stages including nursery and field. This review summarizes the existing information on the taxonomy, distribution, epidemiology, and economic importance of *Pythium* diseases of major vegetables such as tomato, chilli, brinjal, cucumber, capsicum, cabbage, cauliflower, onion and spinach. Soils, which are damp and poorly drained and nursery sanitation are always conducive to outbreak and even though the use of only a system of protection leads to increased productivity, it tends to increase the disease pressure by providing high humidity and limited drainage. Cultural practices and resistant cultivars, biological agents like Trichoderma and fluorescent pseudomonads, and chemical seed treatments are all looked upon with a critical eye on their effectiveness and constraints, especially the development of metalaxyl-resistant populations. The review proposes that there is no single tactic that would provide lasting control and that the most realistic way ahead is by integrating disease management which is based on proper molecular diagnosis and good nursery hygiene. Continued species-level surveillance gaps in the vegetable growing areas and field performance of biocontrol agents are cited as the areas of future research focus.

Keywords: *Pythium*, Root rot, collar rot, seed rot, fungal infection etc.

Introduction

Production of vegetable crops plays a major role in food and nutritional security in both tropical and temperate agriculture but vegetable crops are chronically susceptible to soil-borne diseases which attack the crop at the most sensitive stage of its growth. Within these threats, *Pythium* spp. have some bizarre attributes. They are not fungi or even typical bacteria, but are oomycetes, water moulds that perform ecologically similar functions to fungi but have a biochemistry more similar to algae (Ho, 2018). This double life has some practical implications: there are many fungicides that can control true fungal pathogens that are not effective against *Pythium*, and control must rely on knowledge of the biology of oomycotes.

The *Pythium* species are widely distributed in agricultural soils and survive as saprophytes on crop residues between cropping periods and become pathogenic when a susceptible host is present and the soil is in an appropriate moisture regime (Kucharek & Mitchell, 2000). Seedlings are particularly susceptible due to the very open cell wall and cuticle, which is typical of the genus, and the fast growing hyphae and motile zoospores. The outcome is 'damping-off'-a symptom complex which can kill a nursery bed in days and has been known for more than 100 years as an important limiting factor in vegetable establishment.

This review consolidates the taxonomic, epidemiological and management literature on the *Pythium* diseases of vegetables. It considers the biology of the pathogen and the factors which influence the expression of the disease in various crops, reviews the available management tools for growers and their limitations, and highlights areas of research need. It has not been possible to provide a simple list of management strategies, as the comparative reliability of the various techniques under field conditions has been discussed in relation to the technique.

Taxonomy and Biology of *Pythium* Species

Pythium is an Oomycete in the kingdom Stramenopila, which is an evolutionary outlier to true fungi despite superficial similarities in growth form (Ho, 2018). Most of the species of the genus were thoroughly described by van der Plaats-Niterink (1981), whose monograph defined eighty-five species using mostly the morphology of sporangia and oogonia and has become the taxonomic standard by which much later work has been compared. The primary diagnostic characters have conventionally been the sporangial shape (filamentous or globose) and the ornamentation of the oogonial wall, which may be inconsistent among isolates and are generally interpreted by experts.

Morphological taxonomy by itself has not been sufficient to clarify the actual diversity of the genus. Molecular phylogenetic studies based on the internal transcribed spacer region of ribosomal DNA have since rearranged *Pythium* into a few major clades which do not necessarily fit well with the older morphological classifications, and also have found that some named species are probably synonymous with other genetically distinct clades being undescribed (Lévesque & de Cock, 2004). The practical implications of this taxonomic flux to plant pathologists are that a species name used decades ago using the morphological system might not perfectly match the

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way the organism would be placed in a modern taxonomic system. *Pythium* species are biologically aseptate, coenocytic, and grow rapidly by growing through soil and root tissue. The asexual reproduction is carried out by the sporangia which contain biflagellate zoospores able to move chemotactically in the direction of root exudates, a characteristic that contributes to the high efficiency of the pathogen in exploiting waterlogged or saturated soils. Sexual reproduction gives rise to oospores with thick walls that can remain in the soil over long periods without a host and this is what gives *Pythium* diseases such a hard time to get rid of in infested fields (van der Plaats-Niterink, 1981). A number of them, such as *Pythium aphanidermatum*, *Pythium ultimum, Pythium irregulare, Pythium myriotylum*, and *Pythium debaryanum* are commonly involved in vegetable disease epidemics, but their relative significance changes based on climate, cropping system, and geographic location.

Distribution and Economic Importance

Pythium diseases are found in all vegetable crops grown in open fields, including subtropical and tropical crops, as well as hydroponic and protected crops in temperate greenhouses. *Pythium* aphanidermatum was first recorded as the cause of a serious damping-off of cabbage, cauliflower and knolkhol in early surveys in South Asian vegetable crops in the mid-twentieth century (Mahmud, 1950). A diverse community of *Pythium* spp. has been observed in temperate greenhouse floral and vegetable crops (Moorman *et al.*, 2002), depending on the crop rotation, irrigation source and substrate used.

The economic impact of *Pythium* diseases is difficult to measure accurately because losses are not only due to the death of the seedling, but also to subclinical root infection that may cause a reduction in vigour and yield without producing any noticeable symptoms above ground. A recent thorough review of *Pythium* damping-off and root rot in Capsicum annuum reports that the disease is reported all over the world and can result in significant losses of stands in field and nursery crops, and is exacerbated by the fact that once an outbreak occurs, chemical control is difficult. In the protected culture, the recirculating irrigation system can rapidly spread zoospores throughout the greenhouse; thus, if there is a single source of infection for a growing cycle, the infection can easily progress to a system-wide epidemic as has been observed in hydroponic cucumber crops (Zhao *et al.*, 2000). Hence it is more apt to consider the economic impact of *Pythium* as a variable, and often underappreciated, reduction in productivity that increases as the chance that the pathogen exists increases.

Disease Symptoms and Diagnosis

The clinical manifestation of *Pythium* disease is extremely dependent on the level of the host development when he or she gets infected. Pre-emergence damping-off happens when the pathogen infects seeds or radicals before they emerge to the soil surface causing a soft and watery decay which does not germinate at all; growers normally assume that this is due to poor seed viability and not the disease (Kucharek and Mitchell, 2000). Post-emergence damping-off is more visually striking: seedlings develop normally, but begin to develop a water-filled constricted lesion at the soil line, which leads to the stem collapsing, usually a few days after the first symptoms have been noted.

Infection in older plants is more likely to be in the form of root rot and collar rot, as opposed to a complete collapse. The affected roots become brown to black, soft and easily sloughed, and the entire root system becomes a stubby discoloured stump which is unable to promote normal uptake of water and nutrients. On the surface, this becomes stunt, drying out in the damp atmosphere where it would not otherwise symptomatize, and loss of yield that is all too readily ascribed to other factors unless the root system is considered directly.

Due to the resemblance of symptoms caused by a number of other soil-borne genera, in particular, Fusarium, Rhizoctonia, and Phytophthora, visual observation cannot be the basis of accurate diagnosis. Conventional identification relies on isolating the pathogen on selective media and the morphology of sporangial and oogonial morphology, which is a slow method that utilizes taxonomic expertise that is becoming less common (Schroeder *et al.*, 2013). This situation has improved significantly with the help of molecular diagnostics. Many vegetable pathosystems, such as a set of primers that differentiate between the *Pythium* species that cause cavity spot in carrots and allows them to be directly detected in soil samples without prior isolation, have been developed using PCR assays of species-specific regions of the ribosomal DNA (Klemsdal *et al.*, 2008). These assays, along with quantitative real-time PCR systems that can estimate the inoculum density in soil, can now be used to identify the causal species with a specificity that is unmatched by morphological methods, although cost and availability continues to limit their use in most production areas (Schroeder *et al.*, 2013).

Epidemiology and Disease Development

The cycle of *Pythium* disease starts with the survival of oospores or fragments of the mycelium in the soil or material left by the crops between crop cycles. Exuded sugars and amino acids induce the germination of resting propagules when a vulnerable root or seed in the vicinity approaches, and the release of motile zoospores that swim towards the host on a chemical gradient under under-saturated conditions. This stage of the zoospores is the main cause by which the epidemics of *Pythium* are so closely associated with soil moisture: standing water or wet soil pores are the continuous aqueous film on which zoospores move and disease outbreaks are usually intensely associated with heavy rainfall, over-irrigation, or poor drainage (Kucharek and Mitchell, 2000).

Moisture and temperature are known to interact in the formation of epidemic intensity, but this is species-specific, in contrast with the consistency of the genus. Detailed sugar beet (a crop sharing its main damping-off pathogens with much of the vegetables) demonstrated that plant survival and dry weight decreased with soil temperature and matric potential, which suggested that disease pressure increased with increased warmer and wetter conditions and the biocontrol bacterial treatment of the seed dropped under the same conditions (Schmidt *et al.*, 2004). This observation exemplifies a more general epidemiological rule: the environmental factors that are conducive to the activity of pathogen often also reduce the effectiveness of protective seed treatment, providing a small range within which the effectiveness of control measures is predictable.

The secondary spread in a crop happens not only by movement of soil water that may contain zoospores originating in an infected patch into the rest of the healthy plants but also through cultural practices like irrigation equipment, trays used during transplanting and even foot traffic that can carry infested soil particles between beds. This risk is compounded in the case of a protected and hydroponic system since recirculating nutrient solutions can circulate zoospores through an entire growing system in an hour or so; a weakness that has led to the design of physical barriers like silver-coated cloth filters to pick off zoospores prior to their arriving at the root zone (Zhao *et al.*, 2000). *Pythium* inoculum is likely to survive years when established in soil or growing media by saprophytic growth on organic material even in the absence of a susceptible crop, so that even single-season interventions are unlikely to bring lasting suppression.

Impact of *Pythium* Infection on Vegetable Production

The short-term and most noticeable effect of the *Pythium* infection is the decreased plant stand. Rotting of seeds and pre-emergence damping-off may reduce the germination percentages to the extent that nursery beds have to be resown, and the production schedule must be lengthened and the costs of input multiplied before a single seedling reaches the field. In areas where post-emergence damping-off is dominant, growers might be involved with seemingly healthy stands that soon succumb due to seedlings exceeding a critical density point, as the pathogen is transmitted effectively among tightly spaced and crowded plants.

In addition to the obvious death of plants, the sublethal level of infection of the root weakens the fruit-bearing ability of the remaining plants during the growing period. Infected plants are less efficient in water and nutrient uptake based on damaged root systems, thus in the cases where they do not exhibit the typical collapse of damping-off, they can exhibit reduced vegetative growth, delayed flowering, and reduced fruit size. Combined inoculation of tomato with different Trichoderma isolates was also found to enhance the survival of the plants significantly when compared to controls that were untreated with *Pythium*, which indirectly indicated the magnitude of loss that root rot can cause to yield and fruit set in absence of control (Elshahawy and El-Mohamedy, 2019). Similar effects have been observed in cucumber, with seed and soil inoculation with antagonistic bacteria significantly enhancing seedling survival and fresh biomass compared to untreated, *Pythium*-infested controls, highlighting the extent of productive potential wasted to the pathogen otherwise (Khabbaz & Abbasi, 2014).

Quality parameters are also affected. Transplants that are infected and survive to establish in the field tend to grow unevenly in a planting, making it difficult to schedule harvests and mechanical work. In plants with a high foliage, e.g. spinach, soil-borne pathogens such as *Pythium* species have been known to induce uneven stand establishment which directly limits marketable yield, an issue significant enough to warrant specific assessment of seed and drench strategies within the framework of organic production limited availability of synthetic fungicides (Cummings *et al.*, 2009). These effects, combined, imply that the actual cost of *Pythium* infection is far bigger than the seedlings that die on the spot, including decreased uniformity, maturity, and quality in the surviving crop.

Host Range and Susceptible Vegetable Crops

The species of *Pythium* collectively have a very broad host range, and few vegetable crops are not susceptible at all, but the severity of disease and the causal species predominant in a particular host varies widely. It is often reported that solanaceous vegetables are some of the hardest hit with tomato, chilli and brinjal often mentioned as the most affected crops with damping-off causing significant seedling mortality at nursery stage in various production areas within South Asia and beyond. The same pattern of pre- and post-emergence collapse is repeatedly attributed to *Pythium aphanidermatum* as the main pathogen in these crops with resulting in the characteristic pattern of collapse.

Cucurbits and cucumber, in particular, are also very vulnerable as *Pythium ultimum* can induce the phenomenon of damping-off in young seedlings and root rot in older ones growing in soil, soilless media, and even hydroponics (Zhao *et al.*, 2000; Khabbaz and Abbasi, 2014). Capsicum annuum, including both sweet pepper and hot chilli varieties, is affected by damping-off and root rot to the extent of requiring a specific review, and the pathogen infects crops at nursery, all the way to field-grown plants, and makes it difficult to diagnose and treat due to similarities with other soil-borne diseases.

This is somewhat a different historical situation with the cole crops: cabbage, cauliflower, and knolkhol are some of the earliest vegetables in which *Pythium aphanidermatum* damping-off was first observed, the original description of which dates to the mid-twentieth century and makes these Brassica crops long-established hosts and not new issues (Mahmud, 1950). Although not more commonly mentioned in the literature on biocontrol reviewed here, onion has the same general susceptibility of shallow-rooted, slow-establishing vegetable seedlings to pre-emergence seed rot in cool wet soils common with early spring sowing. Spinach on its part suffers losses of soil-borne seedlings with part being due to *Pythium* species, an issue that is of significant concern to the extent of pursuing systematic analysis of alternative seed and drench treatments in organic spinach production systems where the choice of conventional fungicides is limited (Cummings *et al.*, 2009). Production of floral and vegetable transplanting in green houses presents an additional layer to the host range question, as survey work in Pennsylvania recovered a diverse array of *Pythium* species in greenhouse grown crops, suggesting that covered environments of propagation can be supporting a greater number of pathogenic species than uncovered systems (Moorman *et al.*, 2002).

Factors Favouring Disease Development

The most consistent factor influencing severity of *Pythium* disease in vegetable systems is soil moisture. Any practice or condition that maintains continuous water film throughout the soil profile will significantly enhance the infection pressure, whether it is over-irrigation, poor natural drainage, or high clay soils with small pores space (Kucharek & Mitchell, 2000). Low lying nursery beds and fields with poor surface drainage are hence more susceptible to outbreaks, especially during the first part of the rainy season when temperatures are also favourable for activity of pathogens.

This moisture effect is not independent but is modulated by temperature. Matric potential and soil temperature are both factors that have a significant effect on the outcome of the disease, with increasing matric potential and soil temperature leading to an increasingly poor disease outcome in controlled studies on sugar beet (Schmidt *et al.*, 2004). This dual action can lead to a disease problem while at the same time reducing the effect of a biological or chemical seed treatment, hence the frequent association of warm, humid periods with management failures despite adequate application of a biological or chemical seed treatment.

The use of nurseries and cropping practices further manipulate the risk of disease. High seeding rates provide sufficient crowding to reduce air exchange at the ground level and extend the wetness period of leaf and stem surfaces, thereby favouring the infection and secondary spread of infection from one seedling to the next. Reused growing media, unsterilised irrigation water, and infested transplant trays are examples of persistent sources of inoculum that can be a significant problem and impede otherwise good crop management; the use of re-used substrate in soilless cucumber production is an example where the amount of *Pythium* ultimum inoculum present was enough to necessitate the use of active biological suppression (Liu *et al.*, 2009). The moisture and temperatures mentioned above are aggravated by the continuous growing of susceptible vegetables in the same soil without rotation which allows inoculum to build up in successive seasons and make previously marginal fields increasingly susceptible to severe outbreaks.

Management Strategies

Cultural and Sanitation Practices

The basis of *Pythium* control is still cultural management, exactly due to the fact that the pathogen is particularly sensitive to drainage, sanitation, and crop rotation changes because of its reliance on the presence of free water and organic debris. Raised seedbeds with effective drainage systems, shunning overhead irrigation in favour of drip irrigation, and close control of seed spacing are highly suggested but their efficacy is only achievable when implemented regularly and not occasionally (Kucharek & Mitchell, 2000). Nursery sanitation, such as with the use of pathogen-free growing media, sterilised trays, and clean sources of irrigation water, provides an answer to the fact that a large proportion of early-season inoculum enters a production system via contaminated inputs, rather than via field populations. The cost of these measures is low in comparison to chemical or biological inputs, but they entail a long-term discipline on the part of growers, and are consequently diluted by practice lapses rather than any technical constraint.

Inoculum density can be lowered with time by crop rotation with non-host or less susceptible species, although the wide host range of *Pythium* precludes the success of rotation as does a pathogen that is restricted to a single plant family. Since oospores can survive in soil for years even in the absence of a host, rotation is seldom an adequate measure and is only effective when used with organic amendments and other elements of an integrated programme as opposed to being a single strategy.

Resistant and Tolerant Varieties

The advantage of genetic resistance is that it doesn't need repeated input by the grower if a resistant cultivar is adopted but progress in vegetable crops has been slow. Unfortunately there are only a few cultivars available for commercial use which are resistant to *Pythium*; most cultivars are only partially tolerant. Even when differential susceptibility is present among cultivars as reported for tomato, the level of protection offered might not be as great as with a combination of biological and cultural control measures, and hence cultivar selection is a supplementary rather than primary control strategy.

Biological Control

Biological control has been the subject of significant research interest as an environmentally friendly alternative to fungicide-based control, and a solid body of evidence for the control of *Pythium* in vegetables has emerged. The main mechanism of action of the Trichoderma species is by mycoparasitism, in which the fungus physically invades and degrades the pathogen's hyphae, and by competition for nutrients and sites of infection in the rhizosphere. Elshahawy & El-Mohamedy (2019) found that five *Trichoderma* strains (*Trichoderma harzianum, Trichoderma asperellum, Trichoderma virens, Trichoderma brevicompactum* and *Trichoderma atroviride*) when combined together; decreased severity of root rot and significantly enhanced tomato plant survival when compared with uninoculated plants. In addition, the induction of host defence enzymes (peroxidase and polyphenoloxidase) indicated that the observed protection was not only due to direct antagonism but also to ISR. Similar protection has been observed in cucumber, where *Trichoderma* isolates re-isolated from re-used soilless growing substrates reduced the occurrence of *Pythium* ultimum and enhanced seedling establishment (Liu *et al.*, 2009), and the mode of action of colonisation has been studied in detail: in the case of *Trichoderma harzianum*, it was found that induction of pathogenesis-related proteins in root tissue during the initial stages of colonisation is a common feature of interactions between *Trichoderma* and the host plant, suggesting that the mechanism of action is not simply based on pure antagonism (Yedidia *et al.*, 2000).

Biological control using bacterial antagonists can be considered as an adjunct method. Fluorescent pseudomonads used as seed treatment resulted in significantly lower disease incidence of both tomato and hot pepper when compared to the untreated seed (Ramamoorthy *et al.*, 2002). Comparisons of *Bacillus spp*. vs. *Pseudomonas spp*. using cucumber and sugar beet damping-off have yielded some success with Pseudomonas spp. in general over *Bacillus spp*. and also in some cases showing the importance of delivery method; seed treatments alone have often failed when drench or peat-carrier applications have shown success (Georgakopoulos *et al.*, 2002). In addition, in field-relevant experiments, the growth inhibitory effect of Pseudomonas fluorescens decreased with increasing soil temperature and moisture, which are the conditions when there is the greatest pressure from *Pythium* disease in the field, indicating an important practical limitation: biocontrol agents are often least effective when they are needed most (Schmidt *et al.*, 2004). However, isolates collected from agricultural soil and developed as peat or talc based formulations have been found to be consistently effective in suppressing cucumber damping-off and root rot, and may be more effective than generic commercial products formulated under different environmental conditions (Khabbaz & Abbasi, 2014).

However, with all this evidence available, biological control is not a consistently effective alternative to other management strategies. Efficacy depends on formulation, time of application, soil type and the environmental factors listed above, and biocontrol agents are most effective when they are one part of an integrated as opposed to a sole stand-alone approach to cultural sanitation and, when required, chemical control.

Chemical Management

Historically, systemic fungicides against oomycetes, with metalaxyl and its anthemeric analog mefenoxam being the most commonly used, have been used to provide economical and efficacious control of *Pythium* seed rot and damping-off in a broad spectrum of crops. This dependence on one active ingredient group has, but, yielded foreseeable results. Metalaxyl-resistant populations of *Pythium ultimum* have been observed in highly treated production areas, and the resistant isolates have been found to be consistently resistant across several generations and with no apparent fitness cost, indicating that resistant populations remain in the field despite the lack of further selection. In areas where resistance has become established, growers were forced to use other active ingredients like ethaboxam, which are commonly used with metalaxyl to control populations with both sensitive and resistant genotypes, but in turn, this approach increases the cost of inputs and relies on the fact that resistance to the newer chemistry will not emerge.

Outside the resistance issue, seed treatment formulations more generally have practical limitations involving spectrum of activity, as no single product is likely to offer sufficient control over the entire repertoire of seed-rotting and damping-off pathogens found in a particular field, a constraint that has led to systematic testing of combination and alternative seed treatment regimes in vegetable crops (Mancini and Romanazzi, 2014). Management based on chemicals is thus a mandatory requirement, especially in high value nursery production where minimal or no losses can be afforded, but the sustainability of the practice over time is based on alternating between fungicide groups and using non-chemical strategies instead of depending upon a single compound.

Integrated Disease Management

As the evidence provided above has shown, all evidence tends to lead to one conclusion: there is no single management strategy that is reliable in managing *Pythium* disease under all the conditions that are faced in commercial vegetable production. Cultural sanitation does not eradicate inoculum pressure, but only reduces the extent and availability of resistant cultivars, biological control agents are not as effective in the conditions that are exactly the warm and wet conditions that promote the pathogen, and chemical control is becoming more and more undermined by resistance. A combination of correct diagnosis, sanitation, proper scheduling of drainage and irrigation, judicious use of biological agents and a target application of fungicides only where necessary, therefore, is the most defensible approach at the moment, and is specifically suggested in recent extensive reviews of *Pythium* disease in vegetable hosts like capsicum.

Future Research Priorities

However, there are several knowledge gaps that limit the development of *Pythium* management. Many vegetable growing areas are not routinely monitored for the presence of these species, and diagnostic duties for the species are only partially carried out in the temperate greenhouse environment, where the taxonomic diversity reported by the temperate greenhouse surveys has not yet been similarly documented (Moorman *et al.*, 2002). The availability of species-specific PCR assays that have been validated with carrot cavity spot pathogens such as the *Pythium* species would make it more precise for growers and extensionists to correlate management recommendations with the actual species that may be present in a particular field (Klemsdal *et al.*, 2008).

There is also a need to examine the field performance of biological control agents, as biological control of X. o'teesii by *Pseudomonas fluorescens* is known to be affected by temperature and moisture, and thus highlights the general uncertainty as to how well the results achieved in the greenhouse or growth chamber can be extrapolated to consistent field protection (Schmidt *et al.*, 2004). Research on formulation to maintain the stability of biocontrol agents under the warm and saturated conditions favoring the activity of *Pythium* could be valuable in bridging this gap. Fungicide resistance monitoring should be continued too, as resistance to other chemistries like ethaboxam is not yet common, but has been detected in some *Pythium* species, and could otherwise undermine the value of newer seed treatments if unmanaged. Lastly, breeding for some residual resistance into vegetable cultivars, particularly in solanaceous crops and cucurbit crops where losses are highest, underutilized compared to the effort that has gone into biological and chemical tactics.

Conclusion

Pythium species still stand out as a huge and largely underestimated peril on vegetable growth, as they strike at the most sensitive stage of crop growth and continue to affect crop growth long after so-called seedling death to include lowering of vigour and maturity, as well as the quality of yield. They are a hard-to-find target due to their taxonomic complexity, broad host range, strong reliance on soil moisture and temperature, which renders them a continuing challenge to any single control strategy. As the management literature discussed in this paper has shown, cultural sanitation, resistant germplasm, biological control agents, and chemical seed treatments can all play their own role in the suppression of disease, although each of them has its own set of obvious constraints that manifest themselves most explicitly at the time of the greatest disease pressure. A unified strategy incorporating these strategies, based on proper molecular diagnosis and sensitive to local environmental factors, presents the most realistic hope of long-term protection of vegetable crops against this versatile and invasive pathogen.

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