Article

Susceptibility study of avocado varieties to the attack of mistletoe in Michoacán

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Abstract

The infestations caused by parasitic mistletoe plants, which attack wild and cultivated perennial plants of Mexico, represent one of the main limiting factors of production and are the cause of high mortality rates in plants. The objective was to study the susceptibility of different avocado materials to attack by mistletoe species and identify the genera and species of mistletoe plants that grow on the diversity of avocado trees in the Michoacán avocado region. A fraction of this region was sampled and botanical material from parasitic plants that were affecting avocado trees was collected. In nursery in 'Hass', 'Fuerte' and 'creole' avocado plants, infestations were induced with seeds of the different mistletoe species collected in avocado trees *in situ*. Three seeds were placed per plant and by an experimental design of complete blocks at random, with six repetitions; the response was observed during one year, in terms of the rate of germination and development of endophytic tissue of the hemiparasite on the infested plants. There were three genera and four species parasitizing avocado trees (*Persea americana* Mill. var. *drymifolia*). The results of the induced colonization of the mistletoe, in *Psittacanthus calyculatus*, we found a highly significant statistical difference (Pr> F= 0.0024) for the development of endophytic tissue of the parasitic plant on the different avocado materials used. The highest incidence was in 'creole' avocado with 83.3% and in 'Fuerte' avocado it was 66.7% of infested plants; avocado 'Hass' in none of the plants developed the infection.

Keywords: *Persea americana* Mill., Loranthaceae, parasitic plants.

Reception date: October 2018
Acceptance date: December 2018

Introduction

The infestations and development of the different species of parasitic mistletoe plants, which naturally attack a large number of species of wild and cultivated perennial plants of Mexico, represent one of the main limiting factors of production and are the cause of high rates of mortality in species of cultivated fruit plants (mango, citrus, avocado, guava, apple tree, pear, tejocote, walnut, macadamia, etc.), as well as species of natural forest (pine, oak, spruce, acacia, etc.), ornamental plants (jacaranda, tulip, obelisk, etc.) (Owen, 2004; Vázquez *et al*., 2006; Agrios 2008; Siegfried *et al*., 2008; Ishiwu, 2013). Mistletoes, commonly known as graft or mata palo, are aerial plants, hemiparasites of perennial plants, shrubs or trees of a number of gymnosperm and angiosperm species (Bello, 1984; Bello and Gutiérrez, 1985; Rzedowski, 2011).

They are shrubs or sub-shrubs perennial, monoecious or hermaphroditic, with green, yellow, orange or black leaves, simple, whole, sometimes reduced to scales, of variable forms from liguliforms, lanceolate to elliptical or obovate, solitary flowers or arranged in racemose inflorescence, umbela, cimosas, panicles or spikes, actinomorphic with biseriate perianth, of 3 to 6 divisions, green or of striking colors, without differentiation of the calyx and corolla, but sometimes provided with a calculus; the fruit is a fleshy and mucilaginous berry of variable form, with a seed surrounded by an abundant viscid tissue, the seeds to germinate form a specialized adventitious root (haustorio), through which they penetrate through the tissues of branches and stems (cortex) of the host plants to extract water and nutrients, induce the generation of woody tumors that cause severe damage and even death (Sosa and Tressens, 2002).

The viscous tissue that surrounds the seed of these plants, provides a series of adaptive advantages, given that once dispersed, it adheres strongly to branches and bark of the host. Birds are the main dispersing agents of seeds; the viscous tissue that surrounds the seed, allows it to slide through the digestive tract of the bird, often without losing its consistency and viability, to be excreted in different parts of the host plant forming new colonies (Gómez *et al*., 2011).

This phytosanitary problem represents the second factor of disturbance in the areas of natural forest and commercial plantations (Vázquez *et al*., 2006). Similar impact of damage has been documented in commercial plantations of many perennial fruit species (López and Sanz, 1992; Edagbo *et al*., 2013; Zaragoza *et al*., 2015), as well as in some cases of urban forestry (Arriola *et al*., 2013). At a global level, parasitic plants and in particular mistletoe have been the subject of multiple studies that have allowed to abound in the knowledge of the species that exist, their distribution, biological cycles and the etiology of the disease; however, to date it has not been possible to generate effective management alternatives to avoid damage to host plants (Vázquez *et al*., 2006; Cibrián *et al*., 2007a; Cibrián *et al*., 2007b).

In the particular case of avocado trees, in other countries there have been documented cases of the presence of mistletoe on this species of plant, without specifying the affected varieties or ecological groups (Ishiwu, 2013), there are also undocumented evidences of the presence and attack by

mistletoe in the producing regions of Michoacán, Mexico, but the species of the hemiparasite associated with the avocado tree are unknown, as well as its distribution and damages, which fully justifies this research, in which the objective of studying the susceptibility of different avocado materials to attack by mistletoe species, and identify the genera and species of mistletoe plants that grow on the diversity of avocado trees that cohabit in the avocado producer region of the state of Michoacán.

Materials and methods

For the study was chosen as sampling universe a highly representative fraction of the different climatic conditions occurring in the central producing region of avocado from Michoacán, which was composed of the municipalities of Tingambato, Ziracuaretiro, Uruapan and Nuevo San Juan Parangaricutiro (Figure 1) which, as a whole, make up a compact area that includes a large part, both of the climatic diversity, as well as wild and cultivated avocado materials that exist in the state. The characteristics of the physical environment that prevail in the work area are described in Table 1.

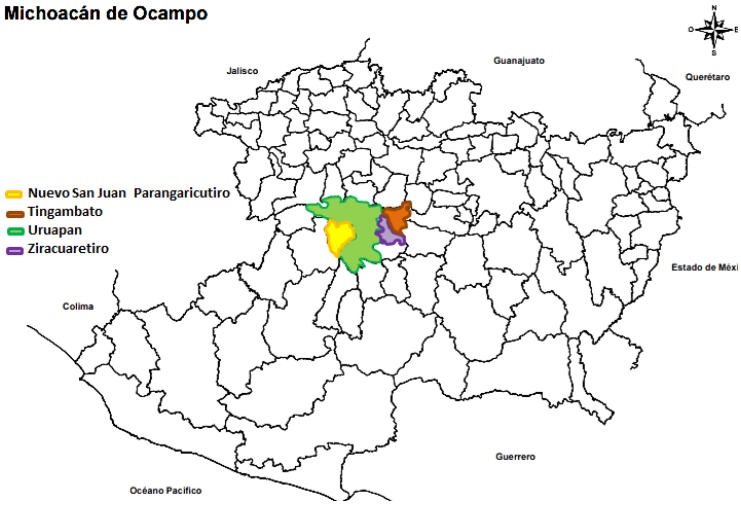

Figure 1. Location of municipalities sampled for collecting mistletoe parasitizing avocado trees of improved and creole varieties (2017).

During 2015, field trips were made to different places in the region of Uruapan, this with the purpose of sampling 'Hass', 'Fuerte' avocado trees and wild or semi-domesticated materials of the Mexican ecotype commonly known as 'creole' In each of the visited sites, we sought to identify trees parasitized by mistletoe plants; and in the cases in which trees were located with the damage, they were georeferenced with a geopositioning (Garmin-20®). To know the characteristics of the physical environment, according to the description shown in Table 1. Additionally, the surrounding avocado trees were visually inspected within a radius of 20 m around the infested tree.

Table 1. Characteristics of the physical environment in the municipalities that make up the avocado producer region of Uruapan, Michoacán 2017.

Physical characteristics	Municipality			
	Tingambato	Ziracuaretiro	Uruapan	Nuevo San Juan Parangaricutiro
North latitude	19º25' and 19º36'	19º21' and 19º31'	19º11' and 19º38'	19º17' and 19º30'
West longitude	101º46' and 101º57'	101º48' and 102º00'	101º563' and 102º24'	102º08'
Altitude (m)	1 600 to 3 000	1 200-2 400	700 to 3 300	1 300 to 3 300
Medium temperature	12 to 22 ºC	16 to 24 ºC	16 to 26 ºC	10 to 22 ºC
Precipitation	1 000 to 1 500 mm	700 to 1 100 mm	800 to 1 500 mm	800 to 2 000 mm
Weather	Temperate subhumid and Tempered humid	Temperate sub-humid, Semi-warm humid, Semi-warm sub-humid, warm sub-humid	Temperate sub-humid, tempered humid and semi-warm sub-humid	Temperate wet and semi-warm sub-humid
Vegetable cover	Pine, oak and bushes	Pine, oak and bushes	Pine, oak and bushes	Pine and oyamel

Source: INEGI (2009). Municipal geographic information booklet of the United Mexican States. Geostatistical key 16058, 16090, 16102 and 16111.

From each of the trees infested by mistletoe, samples of vegetative material and mature fruits of the hemiparasitic plant were collected, deposited inside perfectly labeled paper bags, for later transfer to the herbarium of the National Institute of Ecology AC (INECOL) located in Patzcuaro, Michoacán, where its taxonomic determination and comparison were carried out to later make the description of the species based on the criteria described by Calderón (1979); Oliva (1983); Bello (1984); Bello and Gutiérrez (1985); Hernández (1991); Acosta *et al*. (1992); Gómez *et al*. (2011); Rzedowski and Calderón (2011). The collected specimens are deposited in the herbarium of the Institute of Ecology, AC (INECOL), Patzcuaro, Michoacán.

In the field trips, it was observed that the avocados known as 'creoles' (Figure 2a and 2b) and the 'Fuerte' variety (Figure 2c) are widely attacked by parasitic mistletoe plants; not so 'Hass' avocado trees. To know if this observed situation is the product of chance, or failing that, if the 'Hass' avocado is really tolerant of attack; it is proceeded to establish an experiment in the nursery of the Experimental Field Uruapan, for which we used avocado plants 'Hass', 'Fuerte' and 'creole', with an age of 8 months, which were subjected to infestations induced with seeds of the different species of mistletoe collected from avocado trees in situ. For the above, the study carried out by López and Ornelas (2002) was taken as a reference.

Three seeds were placed per plant and by an experimental design of complete blocks at random, with six repetitions; The response was observed during one year, in terms of the rate of germination and development of endophytic tissue of the hemiparasite on the infested plants.

Special care was taken to avoid the supply of nutrition inputs or pesticides that could interfere with the hemiparasitic. For the registered data, an analysis of variance (Anova) was performed using the proc Anova procedure of the statistical package system for Windows version 9.2 (SAS, 2013). The multiple comparison of the means was carried out by the Tukey test ($p \geq 0.05$), to order the impact of infestation and development of the hemiparasitic plants under treatment.

Figure 2. Trees infested by mistletoe located in places of the avocado producing region of Uruapan, Michoacan 2017. 2a) partial view of a 'creole' avocado tree with severe infestation by mistletoe *Psittacanthus calyculatus*; 2b) plants of *P. calyculatus* in full flowering; 2c) approach to the foliar area of a 'Fuerte' avocado plant infested by mistletoe *Struthanthus venetus*.

Results and discussion

The taxonomic characterization of the mistletoe plants located in 42 places, in the municipalities of Tingambato, Ziracuaretiro, Uruapan and Nuevo San Juan Parangaricutiro, which are part of the central avocado producing region of Michoacan, allowed to register three genera: *Psittacanthus* and *Struthanthus* (Loranthaceae), in addition to *Phoradendron* (Viscaceae) and four species: *Psittancanthus calyculathus*, *Struthanthus venetus*, *Struthanthus condensatus* and *Phoradendron velutinum*, which were found parasitizing on avocado trees (*Persea americana* Mill. var. *drymifolia*) Mexican horticultural race, commonly known as 'Creole' in an altitudinal range that ranged from 1 332 to 2 287 m (Table 2).

Table 2. List of collections made of avocado trees infested by mistletoe. Uruapan Region, Michoacán 2017.

Municipality	Locality	Geographical coordinates		Altitude (m)	Host plant	Mistletoe species
		North Latitude	West longitude			
Nuevo San Juan Parangaricutiro	Hirambosta	19 º 54' 475"	102 º 27' 211"	2 287	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Hirambosta	19 º 54' 475"	102 º 27' 211"	2 287	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Huimbam	19 º 53' 701"	102 º 25' 962"	2 245	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Huimbam	19 º 53' 663"	102 º 26' 012"	2 243	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Hirambosta	19 º 54' 439"	102 º 27' 208"	2 240	Creole	*Phoradendron velutinum*

Municipality	Locality	Geographical coordinates		Altitude (m)	Host plant	Mistletoe species
		North Latitude	West longitude			
Nuevo San Juan Parangaricutiro	Huimbam	19 º 53’ 628”	102 º 26’ 723”	2 225	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Hirambosta	19 º 53’ 510”	102 º 27’ 470”	2 211	Creole	*Phoradendron velutinum*
Nuevo San Juan Parangaricutiro	Hirambosta	19 º 54’ 445”	102 º 27’ 211”	2 210	Creole	*Phoradendron velutinum*
Tingambato	Poblado Tingambato	19 º 49’ 754”	101 º 84’ 936”	1 972	Creole	*Psittacanthus calyculatus*
Tingambato	Poblado Tingambato	19 º 49’ 741”	101 º 84’ 936”	1 972	Creole	*Psittacanthus calyculatus*
Tingambato	Poblado Tingambato	19 º 49’ 753”	101 º 84’ 933”	1 972	Creole	*Psittacanthus calyculatus*
Tingambato	Poblado Tingambato	19 º 50’ 009”	101 º 84’ 759”	1 971	Creole	*Psittacanthus calyculatus*
Tingambato	Centro arqueológico	19 º 49’ 541”	101 º 85’ 822”	1 967	Creole	*Psittacanthus calyculatus*
Tingambato	Llano de la virgen	19 º 50’ 568”	101 º 86’ 704”	1 944	Creole	*Psittacanthus calyculatus*
Tingambato	Centro arqueológico	19 º 49’ 500”	101 º 85’ 872”	1 938	Creole	*Psittacanthus calyculatus*
Tingambato	Centro arqueológico	19 º 49’ 508”	101 º 85’ 865”	1 937	Creole	*Psittacanthus calyculatus*
Tingambato	Llano de la virgen	19 º 50’ 572”	101 º 86’ 739”	1 933	Creole	*Psittacanthus calyculatus*
Tingambato	Centro arqueológico	19 º 49’ 492”	101 º 85’ 890”	1 932	Creole	*Psittacanthus calyculatus*
Tingambato	Llano de la virgen	19 º 49’ 538”	101 º 85’ 778”	1 931	Creole	*Psittacanthus calyculatus*
Tingambato	Centro arqueológico	19 º 49’ 493”	101 º 85’ 883”	1 931	Creole	*Psittacanthus calyculatus*
Tingambato	Llano de la virgen	19 º 50’ 569”	101 º 86’ 781”	1 899	Creole	*Psittacanthus calyculatus*
Ziracuaretiro	Poblado San Ángel	19 º 46’ 176”	101 º 88’ 803”	1 654	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado San Ángel	19 º 44’ 640”	101 º 90’ 566”	1 621	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado San Angel	19 º 45’ 266”	101 º 89’ 389”	1 604	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 550”	101 º 91’ 151”	1 332	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 550”	101 º 91’ 151”	1 332	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 550”	101 º 91’ 151”	1 332	Creole	*Struthanthus venetus*

Municipality	Locality	Geographical coordinates		Altitude (m)	Host plant	Mistletoe species
		North Latitude	West longitude			
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 550”	101 º 91’ 151”	1 332	Fuerte	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 569”	101 º 91’ 113”	1 332	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 464”	101 º 90’ 626”	1 332	Creole	*Struthanthus venetus*
Ziracuaretiro	Poblado Ziracuaretiro	19 º 40’ 901”	101 º 90’ 277”	1 332	Creole	*Struthanthus venetus*
Uruapan	Toreo el bajo	19 º 42’ 701”	102 º 05’ 635”	1 655	Creole	*Struthanthus venetus*
Uruapan	Toreo el bajo	19 º 44’ 624”	102 º 00’ 542”	1 650	Creole	*Struthanthus venetus*
Uruapan	Caltzontzin	19 º 42’ 742”	102 º 00’ 460”	1 634	Creole	*Struthanthus venetus*
Uruapan	Caltzontzin	19 º 42’ 742”	102 º 00’ 460”	1 634	Creole	*Struthanthus venetus*
Uruapan	CE Uruapan	19 º 40’ 722”	102 º 05’ 22.6”	1 600	Creole	*Psittacanthus calyculatus*
Uruapan	CE Uruapan	19 º 40’ 722”	102 º 05’ 22.6”	1 600	Creole	*Struthanthus condensatus*
Uruapan	CE. Uruapan	19 º 40’ 722”	102 º 05’ 22.6”	1 600	Creole	*Struthanthus condensatus*
Uruapan	CE. Uruapan	19 º 40’ 722”	102 º 05’ 22.6”	1 600	Fuerte	*Struthanthus condensatus*
Uruapan	CE. Uruapan	19 º 40’ 722”	102 º 05’ 2.26”	1 600	Creole	*Struthanthus condensatus*
Uruapan	Cd. de Uruapan	19 º 38’ 814	102 º 04’ 7.68”	1 601	Creole	*Psittacanthus calyculatus*
Uruapan	Cd. de Uruapan	19 º 38’ 905	102 º 05’ 4.73”	1 599	Creole	*Psittacanthus calyculatus*

Although there is documented evidence that avocado is included among the natural mistletoe hosts, mainly of the species *Psittacanthus calyculatus* (Vázquez *et al*., 2006; Pérez, 2016), no bibliographic references describe the affected avocado materials. From the results obtained in this investigation, the existence of the Mexican avocado ecotype (*Persea americana* Mill. var. *Drymifolia*), infested by mistletoe, which can be observed in backyards of houses, on roadsides and in an isolated way within some commercial avocado orchards ‘Hass’ which is the totally dominant variety in the orchards of the region. Only two localities were located (Ziracuaretiro and CE Uruapan), with presence of ‘Fuerte’ avocado with mistletoe damage (Table 2).

In the case of avocado ‘Hass’ during the field trips no infestations by mistletoe were found in avocado trees of this variety; this even though in several cases they had neighbors and even crossed the branches with ‘creole’ avocado plants infested by mistletoe. This is the first evidence of the absence of the hemiparasite plant known as mistletoe on avocado trees of the ‘Hass’ variety.

In reference to the 'creole' avocado, trees were found infested by mistletoe, which correspond to three genera and four species of the family Loranthaceae and Viscaceae. Of the four species reported, *Struthanthus venetus* has a greater distribution in properties with semi-warm humid, semi-warm subhumid and warm subhumid climates that prevail in the municipalities of Ziracuaretiro and Uruapan (1 332-1 655 mals) *S. condensatus*, was found in areas with subhumid warm climate of Ziracuaretiro and Uruapan (1 600 masl). *Phoradendron velutinum* was collected from humid temperate zones of the municipality of Nuevo San Juan Parangaricutiro (2 210-2 287 masl). While *Psittacanthus calyculatus* was collected in farms with temperate subhumid and semi-warm humid climate of Uruapan and Tingambato (1 599-1 972 masl). When observing the distribution of these hemiparasitic species in the studied region, it can be deduced that their distribution is subject to their climatic and altitudinal preference.

Finally, two cases of 'Fuerte' avocado were found with infestations by *Struthanthus venetus* and *S. comdensatus* in zones with semi-warm subhumid and warm subhumid climates of the municipalities of Ziracuaretiro and Uruapan at altitudes of 1 332-1 600 m (Table 2).

In the experiment for induced mistletoe colonization, it was found that for the case of the mistletoe species *P. calyculatus*, at the end of the test, a highly significant statistical difference (Pr> F= 0.0024) was quantified for the development of endophytic tissue of the parasitic plant on the different avocado materials subjected to the test. The highest incidence was registered on "creole" avocado, where 83.3% of the plants developed hemiparasitic haustoria on the host stem, in addition to the vegetative development of the aerial part of the mistletoe (Table 3). The 'Fuerte' avocado followed with 66.7% of infested plants; while in the case of avocado 'Hass' none of the plants developed the infection. That is, if there was germination of the seed, but could not develop the haustoria that allowed it to adhere to the host, as shown in Figure 3a, 3b, 3c and 3d.

In reference to the case of the mistletoe *Struthanthus venetus*, a response similar to the previous case was observed, since also at the end of the test a highly significant statistical difference (Pr>F= 0.0202) was presented for the development of endophytic tissue of the parasitic plant on the different avocado materials subjected to the test. The highest incidence was quantified on 'creole' avocado, where 66.7% of the plants developed the infection; followed by 'Fuerte' avocado with 50% infested plants; while in 'Hass' avocado none of the plants developed the infection (Table 3).

Table 3. Mean behavior for infestation induced by mistletoe species on different avocado materials. Uruapan, Michoacán 2017.

Avocado materials	Mistletoe species		
	Psittacanthus calyculatus	*Struthanthus venetus*	*Phoradendron velutinum*
"Creole"	0.8333 a	0.6667 a	0 a
'Fuerte'	0.6667 a	0.5 ab	0 a
'Hass'	0 b	0 b	0 a

Means with the same letter are statistically equal, Tukey ($p \geq 0.05$).

Figure 3. Development of mistletoe-induced infection in avocado plants. 3a) germination of seeds of *Psittacanthus calyculatus* on stems of avocado plants 'Hass'; 3b) development of endophytic tissue of *P. calyculatus* on avocado plants 'creole'; 3c) emission of vegetative shoots of *P. calyculatus* on avocado plants 'creole'; and 3d) endophytic growth of *Struthanthus venetus* on 'Fuerte' avocado plants.

Finally, the seeds of *Phoradendron velutinum* during the time the field trial lasted, did not germinate on the 'Hass' and 'Fuerte' avocado plants, nor did it even replicate the infestation on 'creole' avocado plants, even though seeds of *P. velutinum* were collected precisely from 'creole' avocado trees located in the municipality of Nuevo San Juan Parangaricutiro, Michoacán. This could be attributable to the fact that the environmental conditions prevailing in the sites where the 'creole' avocado trees were collected with natural infestations in the field, correspond to very specific ecological niches located at approximately 2 200 meters above sea level, where the conditions Environmental factors correspond to a temperate humid climate, with temperatures during the year that range from 10 to 22 °C, which favor the presence of rainfall close to 2 000 mm during the year. This contrasts remarkably with the environmental conditions of the experiment site, which is located at 1 600 meters above sea level, presents a sub-humid temperate climate, with an annual rainfall of 1 400 mm and a temperature ranging from 16 to 26 °C.

Of the mistletoe species *Struthanthus condensatus*, it was not possible to obtain seed of the trees observed with infestation in the field, since the degree of damage to the trees was quite severe and the owners determined to tear down the trees at a stage when the mistletoe only presented vegetative development.

Conclusions

All 'Hass' avocado trees sampled in commercial orchards are free from attack by mistletoe.

Avocados 'creoles' can be attacked by mistletoe from one of the species *Struthanthus venetus, S. condensatus, Phoradendron velutinum* and *Psittacanthus calyculatus.*

'Fuerte' avocado trees were found attacked by *Struthanthus venetus* and *S. condensatus.*

In induced infestation on 'Hass' avocado nursery plants, germination of seeds of *Struthanthus venetus* and *Psittacanthus calyculatus* occurred, but in none of the cases haustorial development by the hemiparasitic plant occurred on the host.

In infestations induced with *Struthanthus venetus* and *Psittacanthus calyculatus* on 'Fuerte' and 'creole' avocado nursery plants, there was germination of seeds and development of the endophytic tissue of the hemiparasitic plant on the host.

The seeds of *Phoradendron velutinum* did not germinate in any of the avocado materials subjected to the attack.

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