MORPHOLOGICAL CHARACTERIZATION AND THE GERMINATING POTENTIAL OF *CORONILLA VARIA* L. POLLEN

Silvica PĂDUREANU[1]
E-mail: silvyp27@yahoo.com

Abstract

This scientific paper presented the morphological characterization and characteristics of pollen germination process in *Coronilla varia* L. For morphological characterization have made references on the form, ornamentation exin, size and number of germinative pores/pollen grain. These morphological features are an important tool for diagnosis of plant species. Also we present the features of the pollen germination process of *Coronilla varia* L. Biological material was represented by two population of samples taken from two stationary placed in the surroundings of the Ceahlău National Park: an unpolluted control stationary called Potoci village and a polluted stationary called Taşca-marshalling yard. In this regard have been made of pollen germination tests ”in vitro” in wet rooms "van Tieghem”. Nutritive mediums that served for pollen grain inoculation had different glucide concentrations. The results of experiment presents the minimum, optimum and maximum concentration of sucrose in nutrient media on the pollen of *Coronilla varia* germinate. Determination of the percentage of pollen germinated from time to time (after 1 hour, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours since pollen inoculation in nutritive mediums) allowed determining the pollen germination process dynamics in *Coronilla varia.* The proportions of germinated pollen were surprisingly high in mediums with deficiency glucides elements and glucide concentrated ones, thereby demonstrating the ability to adapt to the wild species.The results can be correlated with ecological plasticity of *Coronilla varia* L. Pollen germination potential in *Coronilla varia* was not influenced by polluted environment, showing that major genes controlled this trait. Therefore, it is very well genetically consolidated. Also, the pollen morphological traits were not influenced by polluted environment.

Key words: *Coronilla varia*, pollen grain, pollen tube, germinating potential, medium

With the studies of pollen morphology of the family *Fabaceae* species, it was concluded that there is little evidence to support the separation of the *Coronilleae* and *Loteae* as two tribes (Díez, M.J., Ferguson, I.K., 1996). These statements are supported by Allan, G.J. and Porter, J.M. (2000), that based on DNA analysis show that the two tribes (*Loteae* and *Coronilleae*) should not be regarded as distinct taxonomic entities. There are other concerns about the molecular phylogeny of species of *Fabaceae*, has reached the same result (Wojciechowski, M.F. et al., 2000).

Coronilla varia L., is relatively self-incompatible. The self pollination and cross pollination experiments proved that pollen germination and pollen tube behavior were the same in flowers from excised stamens and stamens growing on plants. Pollen germination on the stigma and pollen tube penetration of the style occurred within the first 12 hours after self- and cross-pollination. Pollen tubes reached ovaries within 24 hours for both types of pollination (Baluch, S.J. et al., 1973).

The absence of bees (as pollinators) in a population isolated from *Medicago citrina*, determined self-pollinated flowers adaptation, wich still produced more nectar, showing dependence on pollinators. By self-pollination, the amount of fruits and seeds was significantly diminished (Perez-Banon et al., 2003).

Studies of pollen germination of Mediterranean legume species (*Colutea arborescens, Coronilla emerus, Hedysarum coronarium***)** demonstrated the importance of the stigmatic cuticle in reducing levels of autogamy (Galloni, M. et al., 2007).

MATERIAL AND METHOD

The biological material is represented by a vegetal taxon – *Coronilla varia* L., taken from two stationary placed in the surroundings of the Ceahlău National Park: an unpolluted control stationary called Potoci village and a polluted stationary called Taşca-marshalling yard. Last stationary is affected by polluting noxa, which come from the cement factory of Taşca.

From the each stationary we took pollen at the anthesis phase. The pollen was studied as concerns the morphological specific features and the germinating potential. In order to define the pollen morphology, we determined shape of pollen

[1] University of Agricultural Sciences and Veterinary Medicine, Iaşi

grains, exine ornamentation, size of pollen grain and number of germinative pores/pollen grain. For determining the shape of pollen grains and of exine ornamentations, we have used the Tesla electron-scan microscope, at which we took microphotographs.

For determining the size of pollen grains we did micromeasurements at 1000 grains/stationary. We measured the longitudinal and the equatorial diameter. The values obtained were statistically processed, resulting the biostatistics indexes.

For establishing the number of germinative pores/pollen grain, we have done determinations on 1000 pollen grains/taxon/stationary. The method consisted in introducing the pollen in a mixture of concentrated sulphuric acid, acetic acid, methylene blue.

For determining the germinating potential, we have used the so-called van Tieghem "wet rooms". The nutritive mediums necessary for the germination of pollen grains consisted in distilled water, agar 1% and sucrose at different rates: 0%, 5%, 15%, 25%, 35%, 45%, 55%, 70%, 100%, 200%. Thus, ten experimental variants resulted for each stationary. For each experimental variant, we have used 10 "wet rooms". The amount of inoculated pollen per each medium was the same in all cases. Readings at the Hund Wetzlar optic microscope were done at 1, 24, 48, 72, 96, 120 hours since the pollen inoculation on mediums, thus, being established the percent dynamics of the germination capacity.

The germination capacity was expressed as percentage, by reporting the number of germinated grains to total pollen grains.

RESULTS AND DISCUSSIONS

Pollen morphology of *Coronilla varia* L.

Coronilla varia pollen is pale yellow, prolate, tricolporat and exine is reticulate (fig. 1, 2). Length of polar axis has values from 34.5 to 41.4 μm and equatorial axis has values between 20.7 and 27.6 μm (tab. 1, 2). The ratio between the two diameters was around 1.6. These data are consistent with the literature (Díez, M.J., Ferguson, I.K., 1996).

Coronilla varia pollen grains collected from the two stationary have three germinative pores (fig. 3). There is an insignificant percentage of granules with one or two germinative pores (tab. 3).

No difference in shape, size of pollen and number of germinative pores/pollen grain taken from the two stationary.

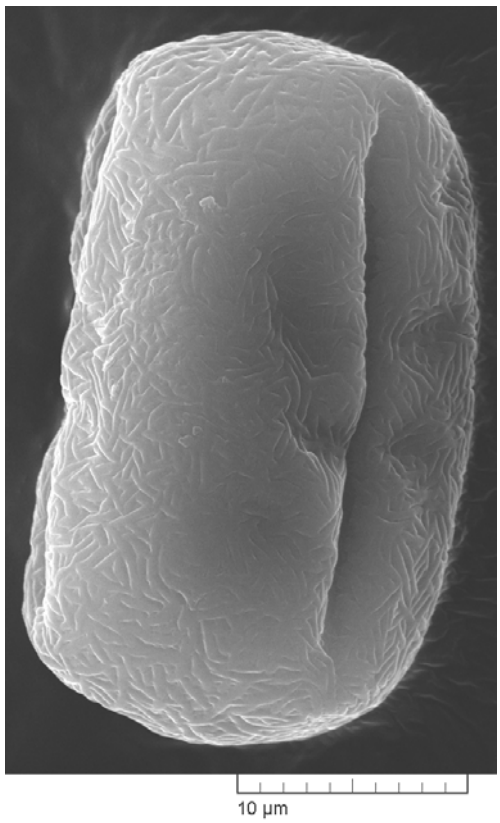

Figure 1 **Pollen grain of *Coronilla varia* L. (Original)**

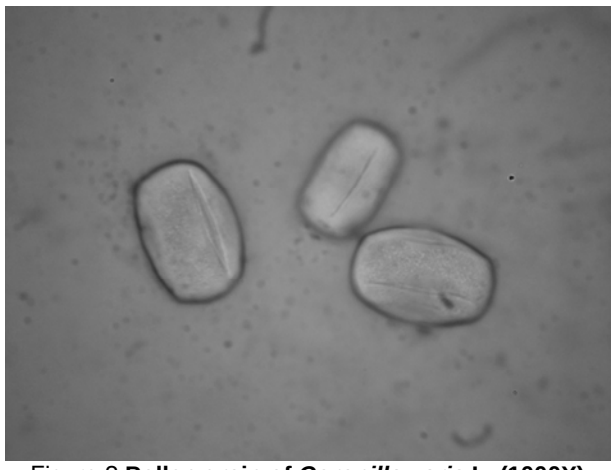

Figure 2 **Pollen grain of *Coronilla varia* L. (1000X) (Original)**

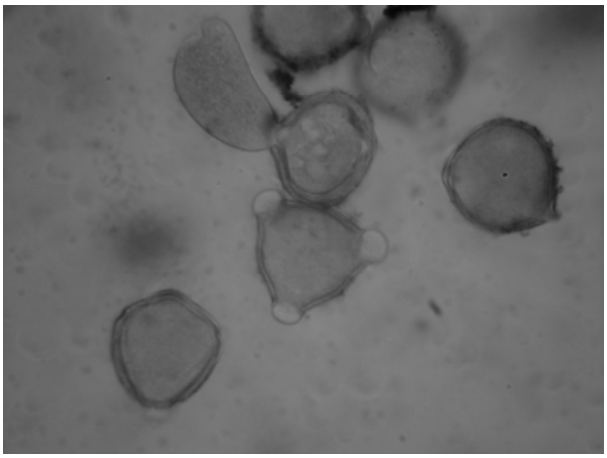

Figure 3 **Germinative pores at pollen grain of *Coronilla varia* L. (1000X) (Original)**

Table 1

Variability of pollen grain size in *Coronilla varia* L. - unpolluted control stationary

Type of diameter	Mean value (µm)	Minimum value (µm)	Maximum value (µm)	Variation height (µm)	S (µm)	S%	$S_{\bar{x}}$ (µm)	Rate high diameter/ equatorial diameter (µm)
high diameter	39.05	34.50	41.40	6.9	2.04	5.22	0.204	1.605
equatorial diameter	24.33	20.70	27.60	6.9	1.27	5.22	0.127	

Table 2

Variability of pollen grain size in *Coronilla varia* L. – polluted stationary

Type of diameter	Mean value (µm)	Minimum value (µm)	Maximum value (µm)	Variation height (µm)	S (µm)	S%	$S_{\bar{x}}$ (µm)	Rate high diameter/ equatorial diameter (µm)
high diameter	39.00	34.50	41.40	6.9	2.038	5.23	0.204	1.609
equatorial diameter	24.24	20.70	27.60	6.9	1.27	5.24	0.127	

Table 3

Number of germinative pores/pollen grain in *Coronilla varia* L.

No. stationary	Mean value ($\bar{X}$)	% pollen grains with … germinative pores		
		1	2	3
Control	2.72	7	14	79
Polluted stationary	2,71	8	13	79

Germinating potential of *Coronilla varia* L. pollen

After an hour since inoculation on nutrient mediums, the germination process was triggered by mediums enriched up to 70% sucrose. It should be noted that the medium without sucrose, germination was possible. Start of germination was similar for the two stationary (fig. 4).

After 24 hours since inoculation, the percent values of control *Coronilla varia* pollen germinability increase significantly al all the variants, and the palette of sugar concentration, which allows pollen germination is larger, reaching 200%. After this time interval of inoculation on medium, the highest rate of germinated pollen was also registered on 15 and 55% sugar mediums (fig. 5). In these cases, the germination was 89-95%. The same situation is found in the case of stationary polluted pollen (fig. 5). After this time interval, germination was also possible on mediums hyperconcentrated in glucidic elements (up to 200% sucrose), in quite high proportions.

After 48 hours since inoculation, in both cases of stationary, we found an increase in the percentage of germinated grains on all the variants of sucrose mediums. On 5%, 15%, 25%, 35%, 45%, 55%, and 70% sucrose mediums, germinated pollen is very high (fig. 6).

After 72 hours since inoculation, there is an insignificant decrease in the percentage of germinaated pollen. But in the medium devoid of sucrose and in the hiperconcentrate mediums (100%, 200%), increased the germinated pollen (fig. 7).

After 96 and 120 hours since inoculation, the percentage values of pollen germination insignificant decrease in all nutrient mediums. The situation is very similar for both stationary (fig. 8, 9).

If we analyse in dynamics the pollen germinating potential of the two stationary, we find that within 24 hours after inoculation, there is a significant jump in growth rates of pollen germinated, and then in the next 48 hours, germinating process continues to grow, but insignificant, on all nutrient mediums (fig. 10, 11). It should be noted that the highest values of pollen germination was possible in mediums enriched with 5%-70% sucrose.

Analyse in dynamics the pollen germination potential found in the process of pollen taken from the two stationary is the same, following the same pace during the 120 hours of observations. The dynamic of the germination potential is the same for the pollen of *Coronilla varia* taken from two stationary, during the 120 hours of observations.

The longest pollen tubes formed on rich medium in sucrose concentration of 15% to 70% (fig. 12, 13).

Pollen germinating very high potential and can be provided by a wide range of nutrient mediums whose concentration of sugar elements may be 0% and even 200%, may be a bioindicator of a special ecological plasticity enjoyed *Coronilla varia*.

Vast area is known worldwide spreading of this species originated in Europe, southwest Asia and northern Africa. *Coronilla varia* spreads fast in an area, become dangerous when invading pastures. A single plant may fully cover 70 to 100 square feet within a four year period.

Coronilla varia is considered a serious management threat to natural areas due to its seeding ability and rapid vegetative spreading by creeping roots. Seeds can remain dormant and viable for over fifteen years.

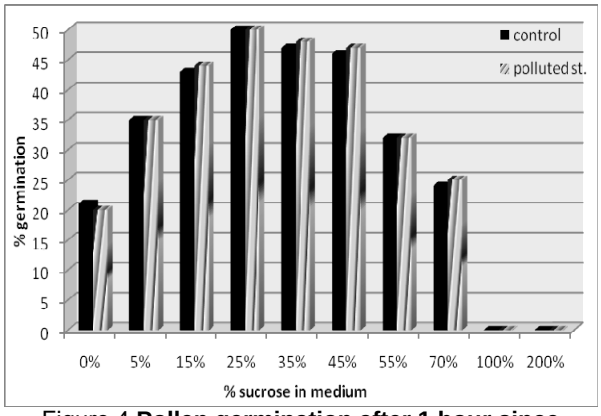

Figure 4 **Pollen germination after 1 hour since inoculation on medium**

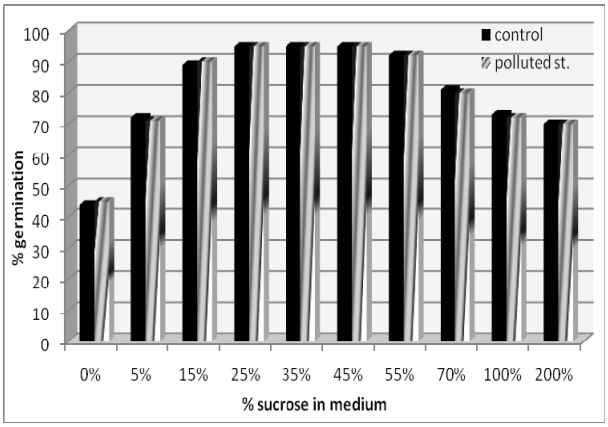

Figure 5 **Pollen germination after 24 hours since inoculation on medium**

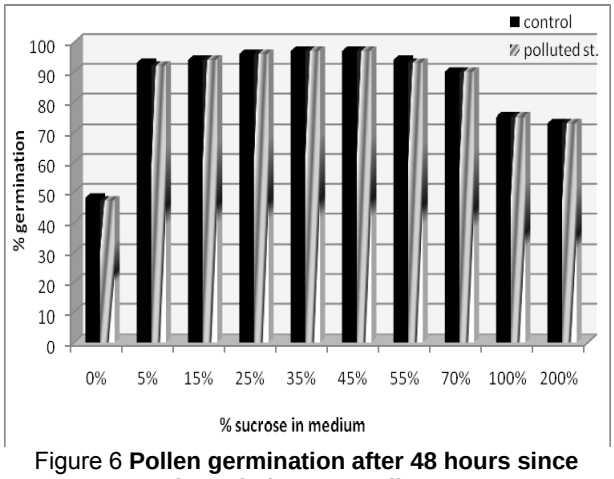

Figure 6 **Pollen germination after 48 hours since inoculation on medium**

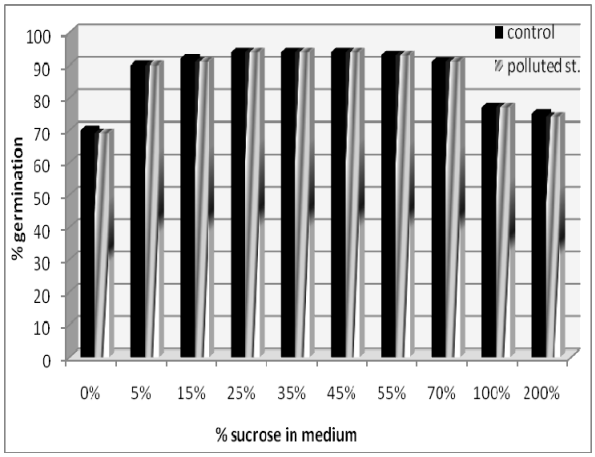

Figure 7 **Pollen germination after 72 hours since inoculation on medium**

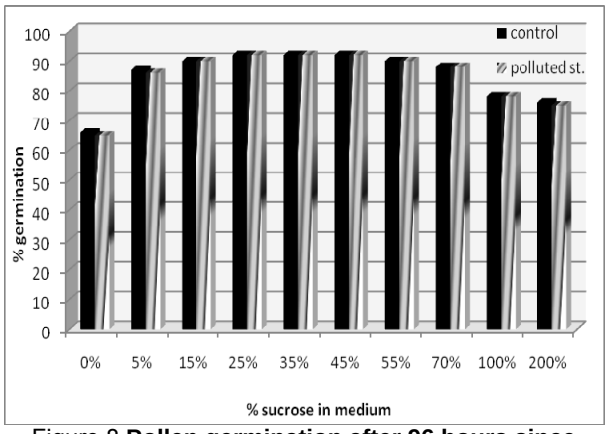

Figure 8 **Pollen germination after 96 hours since inoculation on medium**

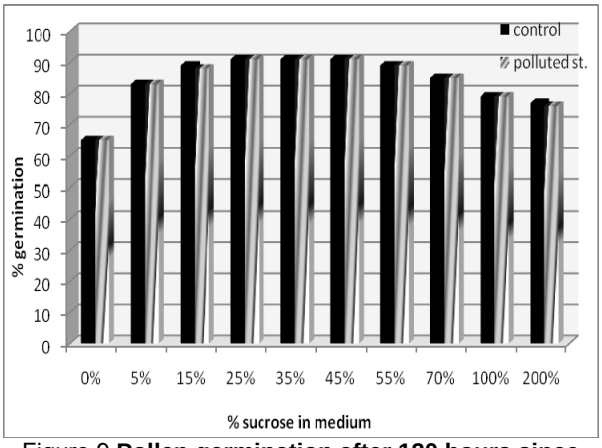

Figure 9 **Pollen germination after 120 hours since inoculation on medium**

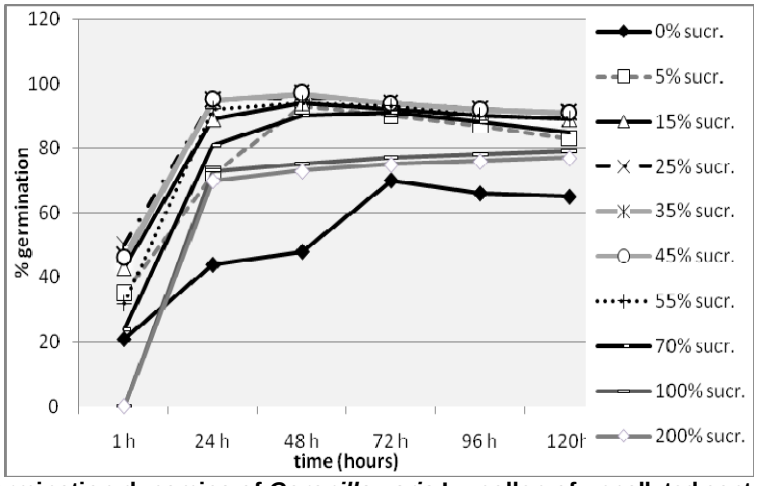

Figure 10 **Germination dynamics of *Coronilla varia* L. pollen of unpolluted control stationary**

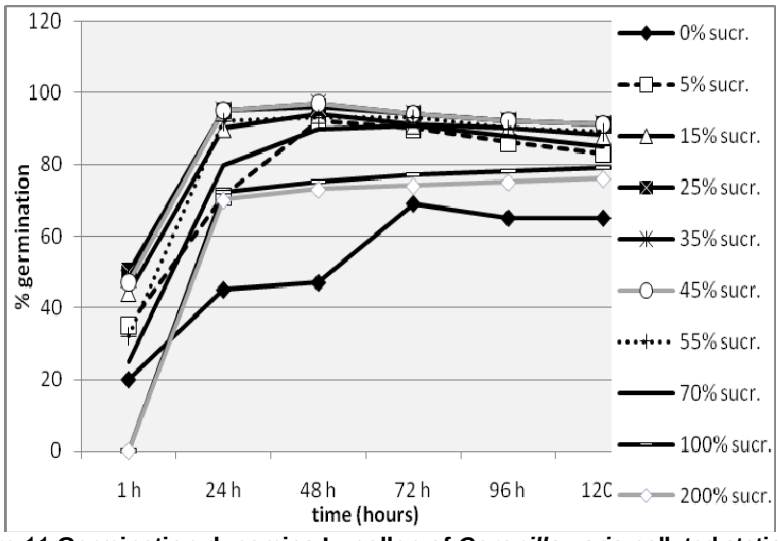

Figure 11 **Germination dynamics L. pollen of *Coronilla varia* polluted stationary**

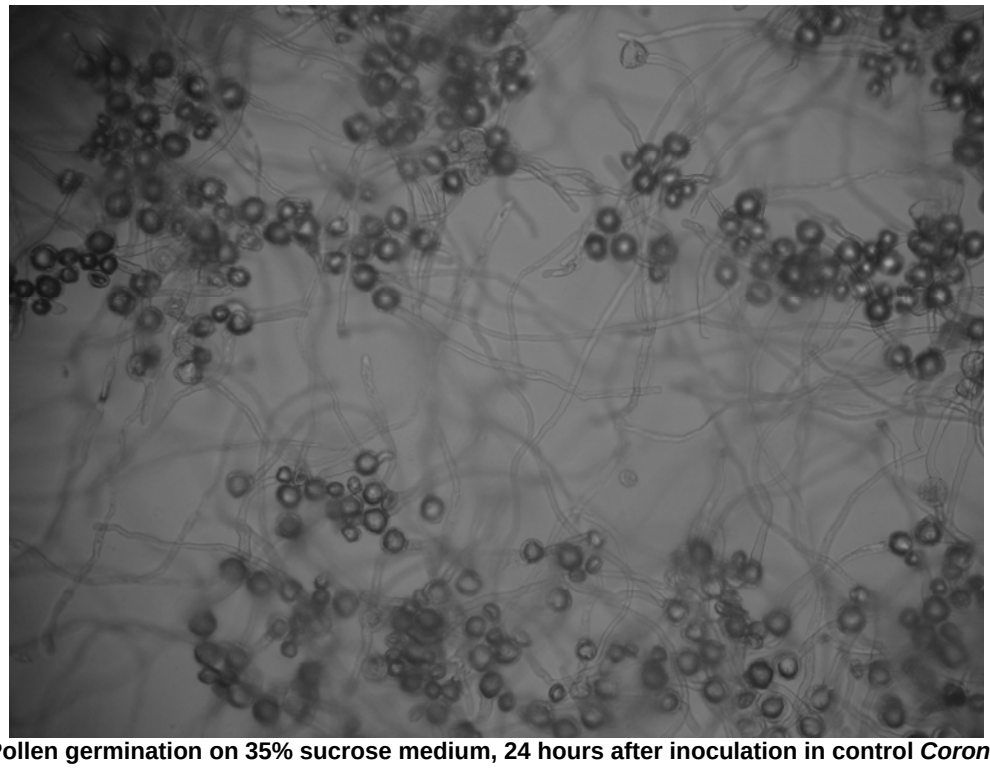

Figure 12 **Pollen germination on 35% sucrose medium, 24 hours after inoculation in control *Coronilla varia* L. (100X) (Original)**

Figure 13 **Pollen germination on 35% sucrose medium, 24 hours after inoculation in control *Coronilla varia* L. (100X) (Original)**

CONCLUSIONS

The shape, the ornamentation of the exine, the size of pollen grains and number of germinative pores/pollen grain from *Coronilla varia* are genetically enhanced characters well.

The germinating potential of *Coronilla varia* pollen, very high, even under a polluted environment, is a proof that and this character is controlled by major genes, therefore, genetically enhanced well.

The highs values of pollen germinating potential of *Coronilla varia* is realized an the nutrient mediums with concentration of 25% to 55% sucrose in the first 24 hours after sowing, but the next few hours, very high values by germinability realized on mediums with 5% to 70% sucrose.

Pollen germination of *Coronilla varia* could be an argument of eco-physiological plasticity and the area of propagation of the species.

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