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## Study of some Pucciniales encountered on weeds in Morocco

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### ABSTRACT

This work is a contribution to the study of the Pucciniales fungi (Basidiomycetes) of Morocco. Surveys in northern Atlantic Morocco have allowed collecting a dozen species of plants (*Euphorbia segetalis*, *E. peplus*, *Carduus tenuiflorus* and *Centaurea calcitrapa*) infected by rust. The different observed symptoms have been described in the laboratory and a microscopic study of spores allowed identifying the responsible pathogens for these symptoms: *Melampsora euphorbiae*, *Melampsora euphorbiae-pepli*, *Puccinia calcitrapae* and *Puccinia carduorum*. The obtained results may help to know the diversification of host Pucciniales and the description of other new species for fungal diversity of Morocco.

**Key words:** Morocco, Weeds, Pucciniales, symptoms, description.

### INTRODUCTION

The Pucciniales, considered as obligatory parasites of plants, they are represented by more than 7000 species<sup>3,8</sup>. The *Puccinia* genus has more than 4877 species widespread in all regions of the world, including 650 species are pathogenic to grasses<sup>2</sup> and others are pathogens on Lily, Umbelliferae and Compositae<sup>35</sup>. Most of these species are heteroxenous, especially those that feed on grasses<sup>35</sup>. In Morocco, *Puccinia* genus is represented by 250 species<sup>23,24</sup> while the *Melampsora* genus is represented by only 18 species<sup>25</sup> which can interfere with different plant species.

Weeds are one of the major obstacles to improving the productivity of crops<sup>40</sup>. They contribute significantly to the production reduction and the quality of agricultural products. Generally, the yield reduction depends, among other things, on the nature and competitiveness of weeds and the crop<sup>6</sup>.

In Morocco, despite the effective use of weeds as livestock feed, because of the losses and the accidents (toxic to humans and / or animals) those they cause, constitute a real and fully justify to the necessity for their control<sup>7</sup>.

Due to poor weed control; weeds annually infest almost 80% of 5 million cultivated hectares in Morocco, wheat and barley<sup>39</sup>. Indeed, inadequate results of the weed control in reduced the national cereal production and generate the import expenditure of these commodities to make up for the deficit<sup>39</sup>.

In Morocco, little information exists in the literature regarding the fungi associated with weeds, especially those belonging to Pucciniales. Surveys carried out along the North Atlantic of Morocco, allowed us to collect four weeds (*Euphorbia segetalis*, *E. peplus*, *Carduus tenuiflorus* and *Centaurea calcitrapa*) infected by four species of fungi responsible for rusts.

In this work, the induced symptoms by these rusts on their hosts as well as the morphological and structural characteristics of these fungi are reviewed and discussed.

### MATERIALS AND METHODS

Surveys carried out in Ouled Berjal and Mamora forest near the town of Kenitra and in a grassy roadside on the way to Tatouft center, near the town of Ksar El Kebir (Northwest of Morocco) during the period

from 29th of May 2011 until 05th of May 2012, allowed to collect a dozen poor sick herbs: *Euphorbia segetalis*, *E. peplus*, *Carduus tenuiflorus* and *Centaurea calcitrapa*. The symptoms are observed on different parts of the host plants.

The description of the symptoms on the hosts was conducted through the use of a pocket magnifier or loupe to better visualize the pustules observed on different parts of weeds.

To study the fungus, a scraping was performed in developed pustules on the leaves and stems of the host plants. Preparations were made for observing through an optical microscope (X 400) especially urediniospores and teliospores to determine the fungus, basing on their morphological characters.

The mounting fluid is tap water, but sometimes added to the preparation a drop of bleach to elucidate the spore wall. The measures of urediniospores, teliospores (at least 50 spores), paraphyses and pedicel were made using an ocular micrometer.

Determining the species responsible for rusts was done through consultation of different determination keys and bibliographic data related to specific studies in Mycology and Plant Pathology<sup>15,17,18,20,28,42,43</sup>.

## RESULTS AND DISCUSSION

### 1 - *Melampsora euphorbiae* (Ficinus & C. Schub.) Castagne (1843)

Species encountered on the March 22<sup>th</sup> 2012 on *Euphorbia segetalis* (L.) along a grassy road and in a field of alfalfa in Ouled Berjal, near to Kenitra city.

*Euphorbia segetalis* (L.) (Fig. 1A), therophyte species, blooms in spring and summer. In Morocco, this plant is found in the lowlands, low and medium mountains, the High Atlas, the Middle Atlas (Bou-Iblane), Northern Atlantic Morocco (Gharb), plateaus of eastern Morocco, the mountains of eastern Morocco (Bni Snassène), the coast of the Mediterranean and the Rif, in the cold semi-arid, subhumid and humid bioclimatic nuances<sup>11</sup>.

The uredia of *Melampsora euphorbiae* (0.25 to 1 mm in length and 0.2 to 0.5 mm in width) are subepidermal, yellow orange color, dispersed or confluent and are located on the underside of the *Euphorbia segetalis* leaves (Fig. 1B).

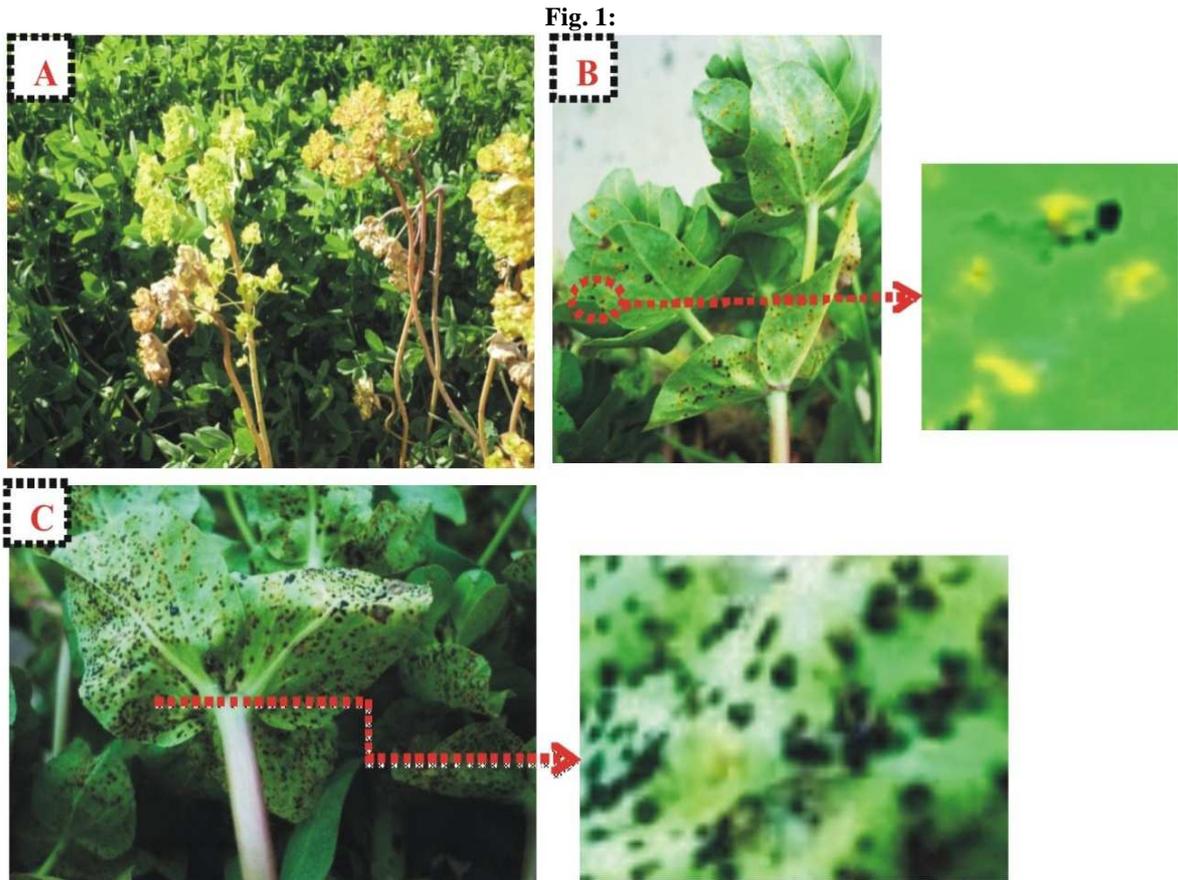
Urediniospores (16.65 to 21.65 µm in length and from 11.65 to 15 µm in width) are subglobose, hyaline, warty and feature a thick wall (2.66 to 5 µm), uncolored and echinulate, and whose germ pore is not noticed (Fig. 2A, 2B, 2C and 2D).

Many subepidermal telia, forming small black crusts were found near uredia on some leaves of *Euphorbia segetalis*. Except for the color, the telia are morphologically identical to uredia (Fig. 1C).

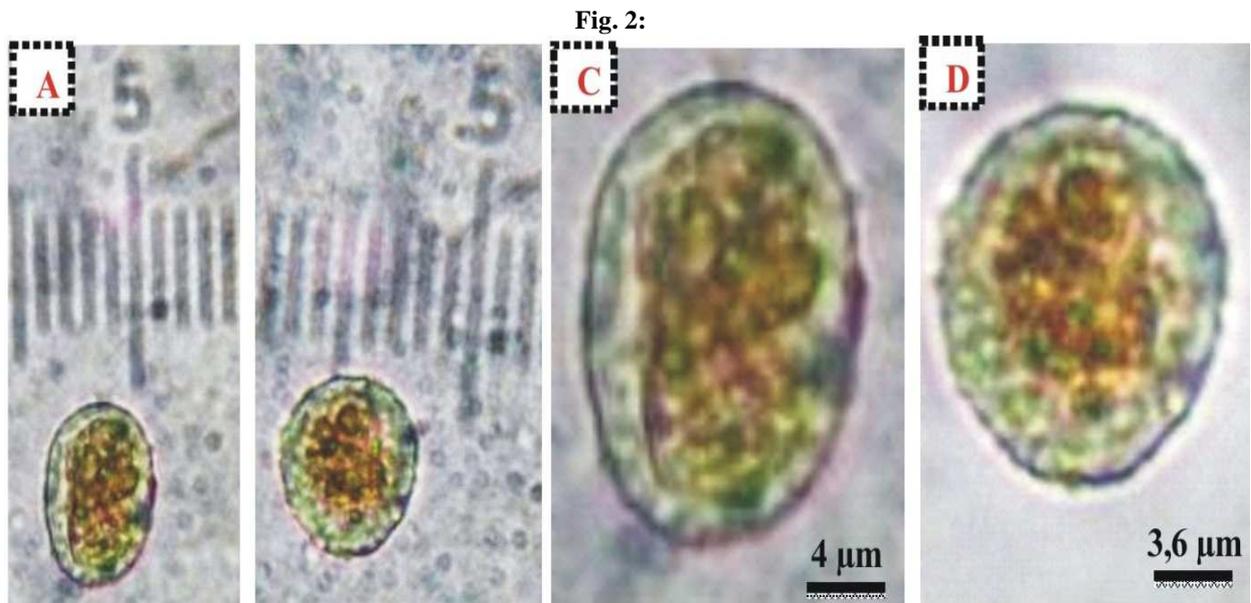
Teliospores are unicellular, sessile, welded, warty, flat and wide at the top, narrow at the basal part and whose dimensions range from 33.30 to 46.62 µm in length and 7.33 to 14.98 µm in width. The epispodium is pale brown, close, from 1 to 1.5 µm thick, but apically thickened to 3 µm (Fig. 3A, 3B, 3C, 3D and 3E). Basidia appear on teliospores.

In Morocco, *Melampsora euphorbiae* was encountered on *Euphorbia peplus* in Tangier<sup>42,43</sup> *E. helioscopia* in a field in Azrou (Middle Atlas) and *E. exigua* slopes of Korifla between Rabat and Camp Marchand<sup>17</sup> on *E. helioscopia*, *E. exigua*, *E. pubescens* var. *genuina* in a valley of Wadi N'Ait Mizan (High Atlas) and *Euphorbia* sp., grassy slope in a degraded cedar forest in Triq Adjir (Middle Atlas)<sup>18</sup> and *Euphorbia exigua*, *E. helioscopia*, *E. peplus* and *E. pubescens* var. *genuina* (no indication of localities)<sup>32</sup>.

*Melampsora euphorbiae*, cosmopolitan species, grows on a large number of species of the *Euphorbia* genus (plants considered as important weeds)<sup>13,20</sup>. This rust may help to reduce the populations of these weeds, and is considered as a means of biological control<sup>20</sup>. The fungus is autoecious<sup>20,30</sup>.



*Euphorbia segetalis* L., in a field of alfalfa (A). Uredia (B) and Telia (C) of *Melampsora euphorbiae* (Ficinus and C. Schub.) Castagne (1843) on the leaves of *Euphorbia segetalis* L.



Urediniospores (A to D) of *Melampsora euphorbiae* (Ficinus & C. Schub.) Castagne (1843) taken from *Euphorbia segetalis* L. (x400).

Fig. 3:



Teliospores (A to E) of *Melampsora euphorbiae* (Ficinus & C. Schub.) Castagne (1843) on *Euphorbia segetalis* L. Details of image B represents a basidium bearing basidiospores. Mounting fluid: chlorinated tap water. Magnification: x400.

*Euphorbia segetalis* is a new host for *Melampsora euphorbiae* in Morocco.

From a morphological point of view; *Melampsora euphorbiae* is indistinguishable from *Melampsora helioscopiae*. Indeed, the structural differences cited by Sydow<sup>36</sup> and Gonzalez Frago<sup>16</sup> lie in the fact that teliospores are longer and narrower in *Melampsora helioscopiae* (III: 40-60 x 7-12 microns) than in *Melampsora euphorbiae* (III: 28-50 x 7-15). The obtained results on our Moroccan harvest, as well as those obtained by Guyot and Malençon<sup>17,18</sup> are consistent with the work Sydow<sup>36</sup> and Gonzalez Frago<sup>16</sup>.

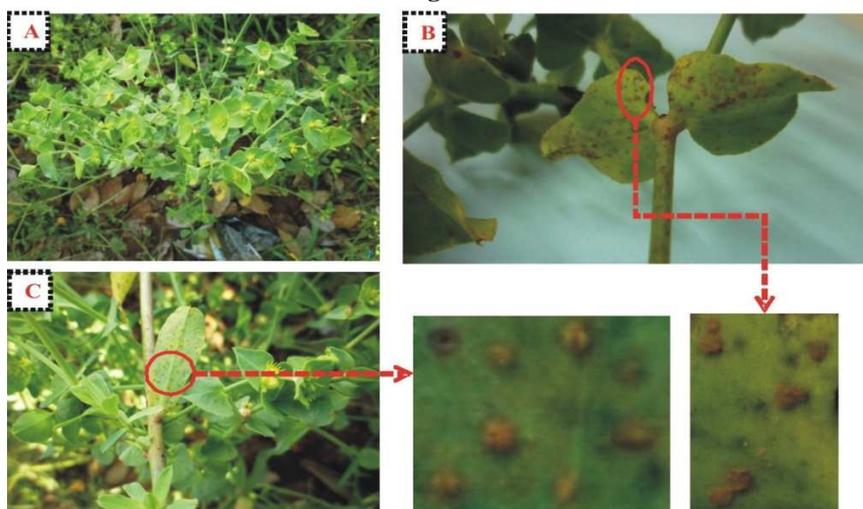
## 2 - *Melampsora euphorbiae-pepli* Muell W., (1907)

Species encountered on the 5<sup>th</sup> of May, 2012 on *Euphorbia peplus* L. in the Maamora forest near to Kenitra city (Northwest Morocco).

*Euphorbia Peplis* (Fig. 4A) is a therophyte species whose flowering is possible throughout the year. This species is found in the plains and in the low and medium mountains, in different bioclimatic nuances: arid, semi-arid sweet, cold semi-arid, sub-humid and humid. It has been reported throughout Morocco, but considered rare in the Sahara of Morocco and the Saharian Atlas<sup>11</sup>. Similarly, *Euphorbia peplis* was newly inventoried in the Mamora forest<sup>1</sup>.

*Melampsora euphorbiae-pepli* develops symptoms that appear as yellow pustules occurring most often in abundance on the lower leaf surface of the support (*Euphorbia peplus*). These sores are rounded, covered with a very thin wall, very close together (sometimes subconfluent) and measure 0.2 to 0.5 mm in diameter (Fig. 4B and 4C).

Fig. 4:



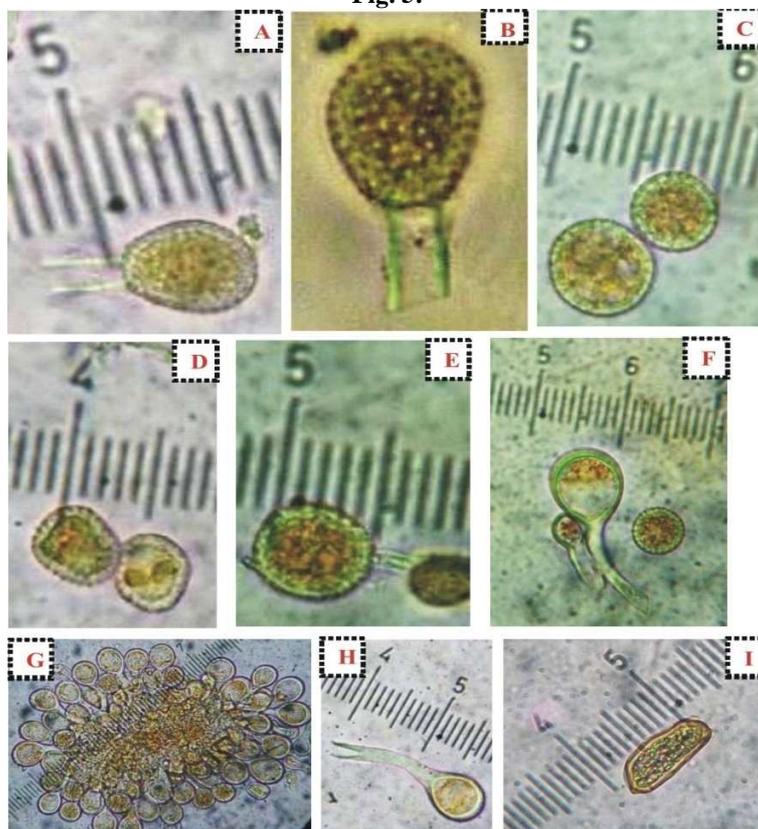
General appearance of *Euphorbia peplus* L. (A). Symptoms (B and C) of *Melampsora euphorbiae-pepli* W. Muell. (1907) on green leaves of *Euphorbia peplus* L.

Urediniospores, measure 20 to 21.65  $\mu\text{m}$  in length and 13.32 to 16.65  $\mu\text{m}$  in width, are unicellular, rough, brownish biguttulate, polymorphic (subglobose, globose or ovoid) and supported by a pedicel. The Pedicel is hyaline or brownish, 4 to 6.66  $\mu\text{m}$  in width and can reach up to 23.31  $\mu\text{m}$ . The wall is colorless, thick (2.66 to 3.35  $\mu\text{m}$ ) and finely wart (Fig. 5A, 5B, 5C, 5D and 5E).

Many urediniospores and paraphyses were observed in combination (Fig. 5F and 5G). Paraphyses are longer (from 53.28 to 59.94  $\mu\text{m}$ ) than broad (from 18.31 to 23.31  $\mu\text{m}$ ), measure from 2.31 to 3.66  $\mu\text{m}$  thick and have a greenish wall (Fig. 5F, 5G and 5H).

Teliospores (8 to 15  $\mu\text{m}$  in width and 33.30 to 40  $\mu\text{m}$  in length) are unicellular, prismatic, sessile (not stalked), greenish brown color, uniform, smooth, thin-walled and has a single germ pore (Fig. 5I).

Fig. 5:



urediniospores (A to E) with paraphyses (F, G and H) and teliospores (I) of *Melampsora euphorbiae-pepli* Muell W., (1907) taken from *Euphorbia peplus* (L.). (Magnification: x400)

*Melampsora euphorbiae-pepli* is rust that has never been reported in Morocco or in *Euphorbia peplus* or in other host plants.

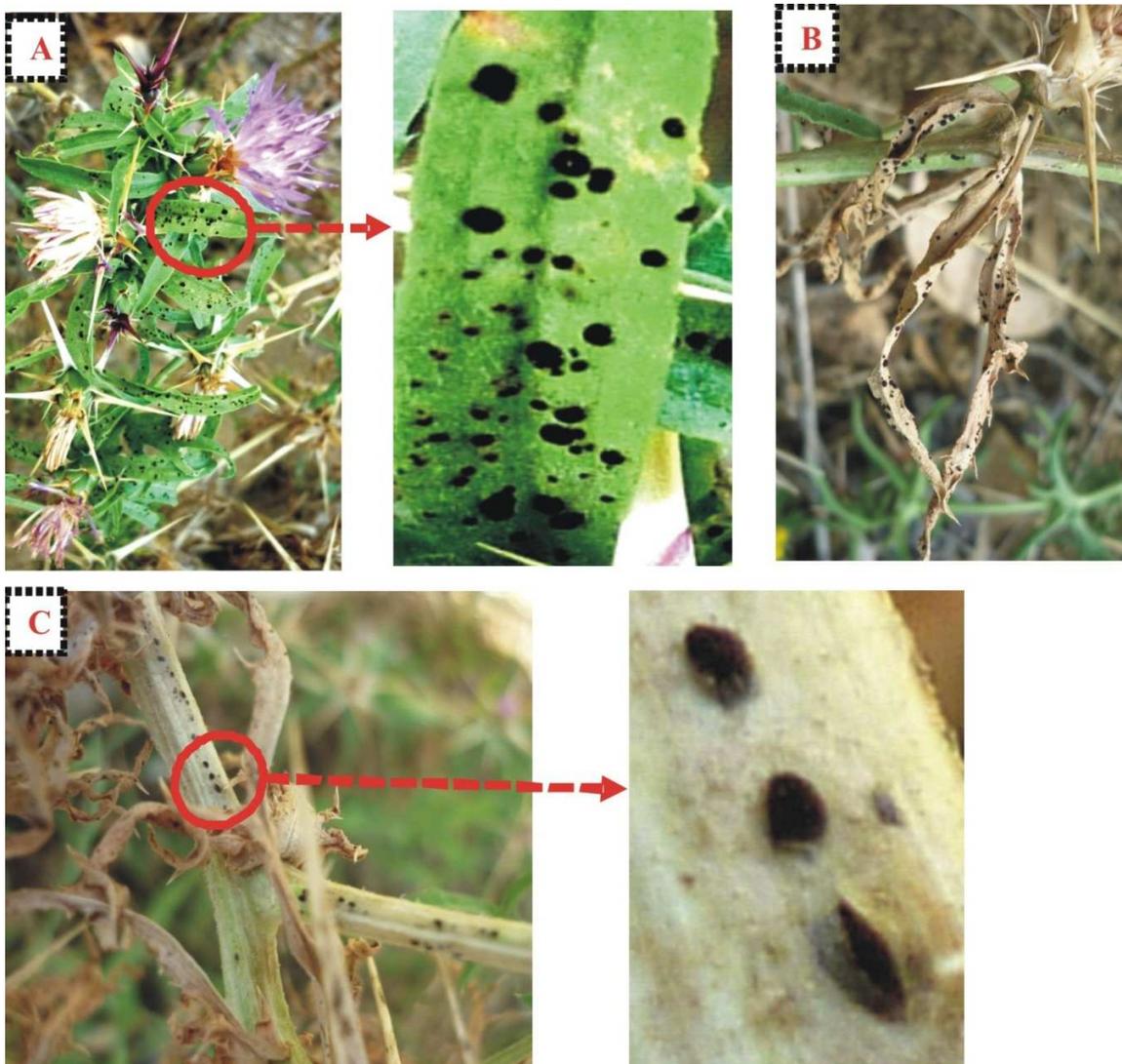
### 3 - *Puccinia calcitrapae* DC.

Species encountered on May 29<sup>th</sup>, 2011 on *Centaurea calcitrapa* L. in a grassy roadside in Oued El Makhazine near the town of Ksar El Kebir.

*Centaurea Caltrop* (*Centaurea calcitrapa*) (Fig. 6A) is a weed of autumn cereals (especially wheat and barley of Chaouia)<sup>37,38,39</sup>. *C. calcitrapa* is a species that grows in border generally moist habitats during the rainy period<sup>19</sup>, as well as loam soils, irrigated, rich in lime and organic matter and showing basic pH<sup>41</sup>. It is a xerophytic species that grows on dry, rocky phosphate land, loamy to silty clay, where barley is mostly cultivated<sup>37</sup>.

On stems and at the upper surface of leaves still green and dried *Centaurea calcitrapa* grow scattered or more or less densely grouped sores. These sores are black, round and oblong, covered with a skin at first and then exploded, powdered, carried by swellings and measure 2 to 4 mm in length and 1 to 1.5 mm in width (Fig. 6A, 6B and 6C).

Fig. 6:

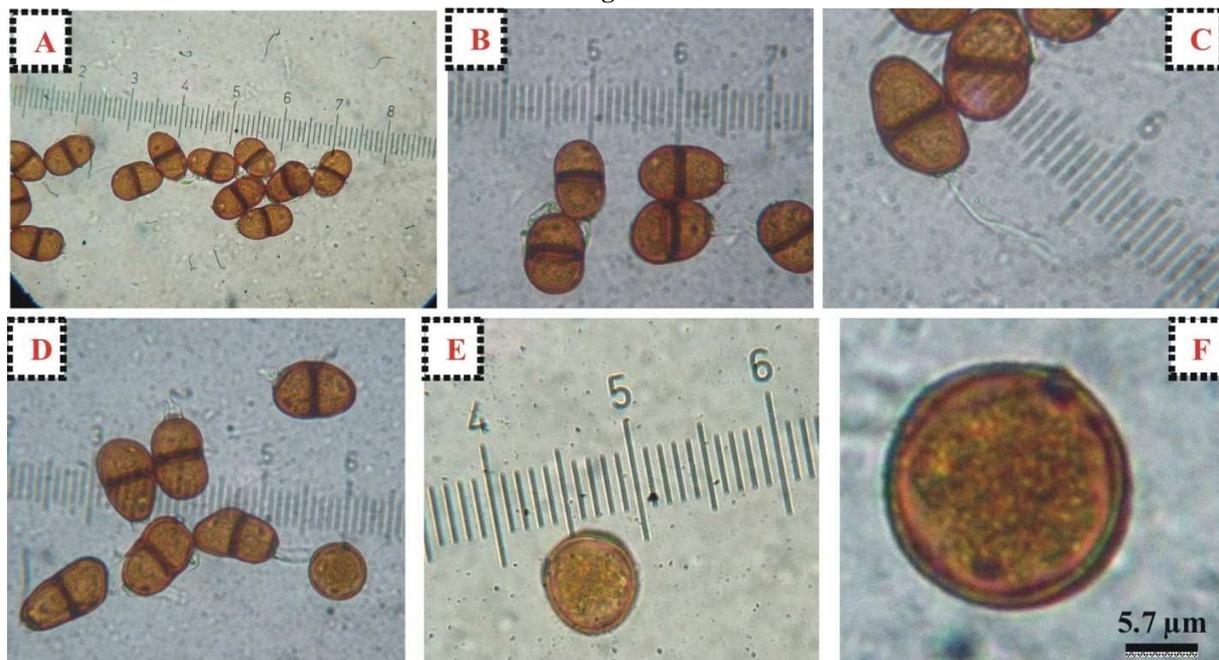


Symptoms of *Puccinia calcitrapae* DC. (1805) on stem (C), green leaves (A) and dry leaves (B) of *Centaurea calcitrapa* L.

Teliospores (29.95 to 33.30  $\mu\text{m}$  in length and from 16.65 to 20  $\mu\text{m}$  in width) are stalked, two-celled, orange, yellow rough and have a subapical germ pore. The two cells of teliospores are asphyxiated, so that the end of the cell is rounded and the lower cell is sometimes slightly narrow. The episporium measures 3  $\mu\text{m}$  in diameter, smooth and colored with brown to light brown (Fig. 7A and 7B). Pedicel hyaline, thick (4  $\mu\text{m}$ ) and can reach up to 36.60  $\mu\text{m}$  in length (Fig. 7C).

Urediniospores (average diameter 23.30  $\mu\text{m}$ ) are globular, yellow orange, warty connected and equipped with one to three germ pores (2 germ pores in most cases); the number of urediniospores is low compared with that of teliospores (due to the late harvest) (Fig. 7D and 7F). The wall of urediniospores is thick (3.65  $\mu\text{m}$ ), brownish and finely and little echinulate (Fig. 7E).

Fig. 7:



spores of *Puccinia calcitrapae* DC, 1805. (A and B) Teliospores; (C) Teliospores provided with a long stalk; (D) Urediniospores with teliospores; (E and F) Urediniospores. Mounting fluid: tap water. (Magnification: x400)

*Puccinia calcitrapae* is an autoecious rust<sup>9</sup>, which has never been reported on *Centaurea calcitrapa* in Northern Atlantic Morocco.

In Morocco, *Puccinia calcitrapae* was reported on *Centaurea calcitrapa* in the banks of the Oued Réraia near Asni (Atlas)<sup>17,33</sup>.

On the world scale, *Puccinia calcitrapae* which was encountered on several species in the *Centaurea* genus, include for example: *Centaurea alperstris* in Poland<sup>29</sup>, *C. balsamita* in Iran<sup>12</sup>, *C. behen* in Iraq<sup>27</sup>, *C. biebersteinii* in Romania<sup>21</sup> and *C. calcitrapa* in France<sup>34</sup>, Spain<sup>14</sup>, Portugal<sup>15</sup> and Pakistan<sup>22</sup>.

#### 4 - *Puccinia carduorum* Jacky (1899)

The encountered species on the 14<sup>th</sup> of April, 2012 on *Carduus tenuiflorus* Curtis (1793), in a grassy roadside on the way to Tatouft center, near the town of Ksar El Kebir.

*Carduus tenuiflorus* is an annual weed that prefers moderate soil, fertility and high areas where rainfall is moderate<sup>10</sup>.

*Puccinia carduorum* develops symptoms represented by sores located in internervure areas of both sides of the still-green and semi dried leaves of *Carduus tenuiflorus*. These sores are powdered, scattered or confluent, surrounded by the debris of a whitish skin and have an oblong shape. They are dark brown to black color depending on the stages of disease development and measure 0.15 to 0.4 mm in width and from 0.25 to 1.2 mm in length (Fig. 8A).

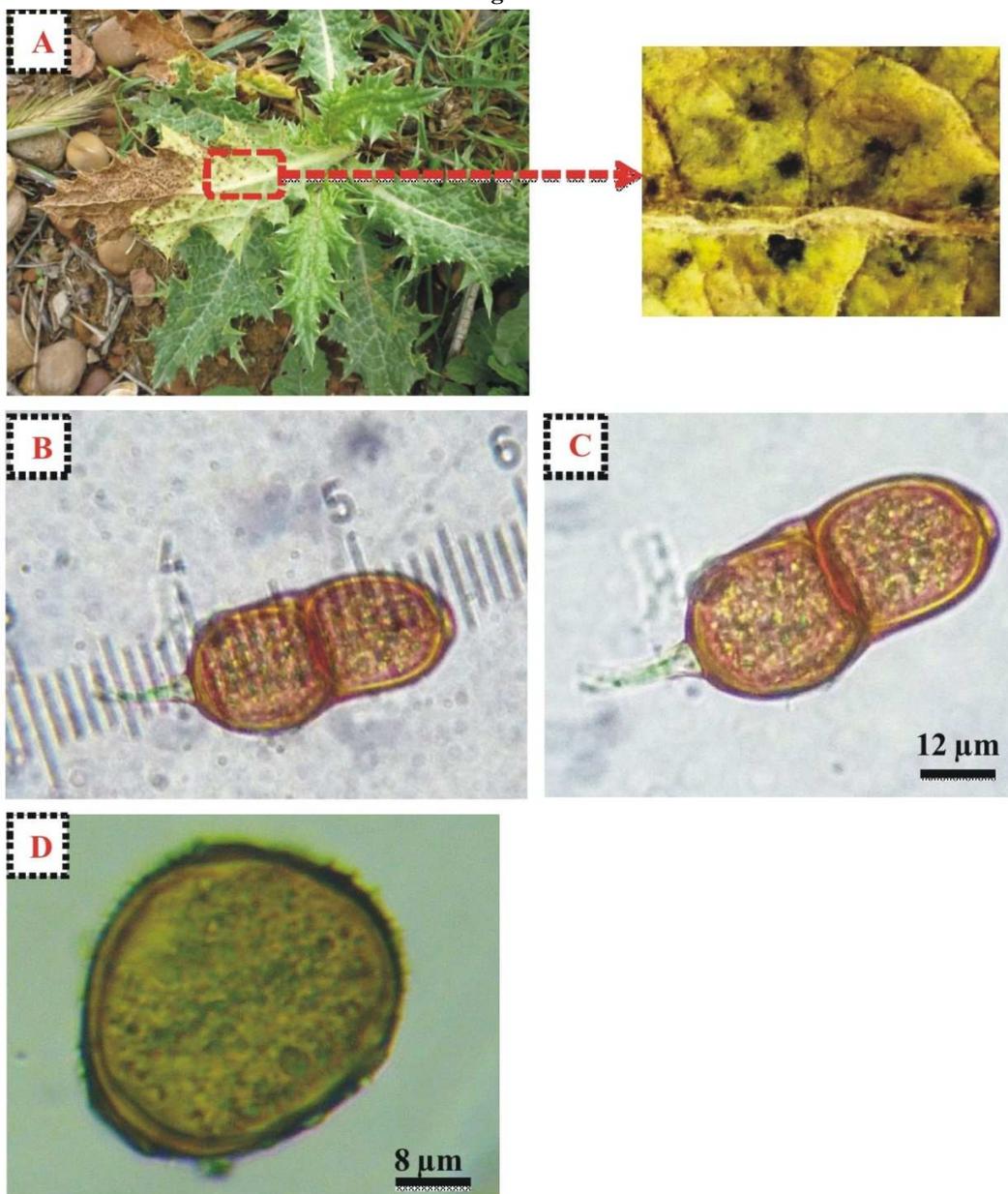
Teliospores (measure from 46 to 53.28  $\mu\text{m}$  and in length from 26.65 to 31.60  $\mu\text{m}$  in width) are bicellular, stalked, rough, ocher brown and have two germinal pores (one at the apical level of the upper probaside and the other is equatorial on the other cell). The wall is smooth, thick (4 to 5.30  $\mu\text{m}$ ) and has a light brown color. Both probasides of teliospores are generally rounded and slightly choked, but some are slightly pointed at the top. Pedicel hyaline measure 2  $\mu\text{m}$  thick and 19.95  $\mu\text{m}$  in length (Fig. 8B and 8C).

Urediniospores are ovoid or subglobose, rough, brown and thick-walled (1,6 to 4  $\mu\text{m}$ ). They are thorny, have 2 to 3 germ pores and measure 22 to 28  $\mu\text{m}$  in diameter (Fig. 8C).

In Morocco, *Puccinia carduorum* was encountered on *Carduus ballii*, *C. chevallierri*, *C. macrocephalus*, *C. myriacanthus* and *C. spachianus* in Targuist<sup>26</sup> and *Carduus ballii*, *C. chevallierri*, *C. leptocladus*, *C. macrocephalus*, *C. meonanthus*, *C. myriacanthus* and *C. spochianus* (unspecified locality)<sup>33</sup>.

These two citations reported the presence of this rust, but they did describe neither symptoms nor biometric characteristics of the fungus. *Puccinia carduorum* is a rust fungus described for the first time on *Carduus tenuiflorus* which can be considered as a new host for this fungus in Morocco<sup>24</sup>.

Fig. 8:



Symptoms of *Puccinia Carduorum* Jacky (1899) on the leaves of *Carduus tenuiflorus* Curtis (1793) (A). Teliospores (B and C) and urediniospores (D) of *Puccinia Carduorum* Jacky (1899). (Magnification: x400)

*Puccinia carduorum*, native to the Mediterranean region, was also reported in Bulgaria and Romania<sup>31</sup>. This autoecious rust produces urediniospores and teliospores on many species of the *Carduus* genus, and can be used as a biological control against several species belonging to the *Carduus* genus<sup>4,5,28</sup>.

### CONCLUSION

The study of four rust fungal species, encountered on four weeds, allowed deducing that:

- \* *Melampsora euphorbiae-pepli* is encountered for the first time in Morocco on *Euphorbia peplus*.
- \* *Puccinia carduorum* is described for the first time in Morocco on *Carduus tenuiflorus*.
- \* *Carduus tenuiflorus* and *Euphorbia segetalis* are new hosts, in Morocco, for *Puccinia carduorum* and *Melampsora euphorbiae*.
- \* *Puccinia calcitrapae* is a new host in Northern Atlantic Morocco (Northwest Morocco) for *Centaurea calcitrapa*.

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