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3 **Physiological Maturity and determination of the**
4 **harvest time of *Vigna unguiculata* L. Walp.**

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8
9 **ABSTRACT**

10 The maturation process of seeds is genetically controlled and involves an organized sequence of physiological changes from the fertilization until the complete independence from the plant. It is recommended that the harvest occurs in the ideal moment, this way the seeds can express their full potential, with maximum dry matter accumulation, reaching high potential of germination and vigor. The objective of this study was to determine the physiological maturity point of cowpea bean seeds (*Vigna unguiculata* L. Walp.), cv. Corujinha, aiming to indicate the best harvesting period, in order to guarantee greater germination potential and seed vigor. Cowpea pods were harvest from the third until the twenty-first day after anthesis, with intervals of three days between the harvests. After each harvest, the following evaluations were carried out: fruit and seed color, number of seeds per pod, pod and seed biometry, pod and seed moisture, water content, germination, germination speed index, length and dry matter of the shoots and roots. At 15 DAA, the seeds and fruits presented light green coloration, with maximum values of length, width, thickness, dry matter, germination percentage and germination speed index, at a vigor level. There was a gradual reduction of water content in the seeds and number of seeds up to 21 DAA. The highest values for shoot and root length were observed at 18 DAA, when seeds and fruits showed light brown color and for shoot and root dry matter at 21 DAA, with brown color. The physiological maturity of cowpea seeds was rapid and occurred between 15 and 21 DAA. The harvest is recommended at 15 days after anthesis, when the seeds present high germination and vigor.

11
12 *Keywords: Cowpean bean, physiological quality, germination, vigor.*

13 **1. INTRODUCTION**

14 The cowpea (*Vigna unguiculata* L. Walp.) is cultivated throughout the North and Northeast of
15 Brazil, where it is considered the main component of the agricultural production of these
16 regions, constituting an important source of income and subsistence for small farmers who
17 practice agriculture. In addition, this crop is used as a staple food for the population, which
18 consumes it in the form of green and dry grains, being very appreciated due to the fast
19 cooking and nutritional aspects, such as the quantity of proteins [1].

20 The difficulty of obtaining seeds of good physiological quality is among the limiting factors in
21 the production of cowpea, since the seeds are one of the main inputs of the agricultural
22 production, where the quality is an important factor to obtain stands of uniform and vigorous
23 plants, directly reflecting the yield [2].

24 To express its full potential, it is essential that the harvest occurs at the ideal moment, with
25 maximum dry matter accumulation, reaching high germination and vigor potential [3].
26 Therefore, the study of the physiological maturation process of seeds is very important to

27 determine the ideal harvest time and, consequently, obtain seeds of high physiological
28 quality [4].

29 The seed maturation process is genetically controlled and involves an organized sequence
30 of physical, biochemical, physiological and morphological changes, from the fertilization until
31 its independence from the plant, these changes also include a set of preparatory steps for
32 the process of germination, which are characterized by the synthesis and accumulation of
33 nutrient reserves [5].

34 Several studies on the influence of physiological maturity on the seed quality and
35 productivity of several crops have been carried out, such as the studies with pepper seeds
36 (*Capsicum annuum* L.) [6] common bean (*Phaseolus vulgaris* L.) [7], ginger (*Sesamum*
37 *indicum* L.) [8] and pumpkin (*Curcubita moschata* Duch) [9]. However, currently, for cowpea,
38 there is little information on the maturation and the ideal harvest period of the seeds,
39 justifying the need to perform this evaluation [2].

40 The objective of this study was to determine the physiological maturity point of cowpea
41 seeds (*Vigna unguiculata* L. Walp.), cv. Corujinha, aiming to indicate the best **harvest** time,
42 in order to guarantee greater germination potential and seed vigor.

43 **2. MATERIAL AND METHODS**

44 **2.1 Experimental Location**

45 The field experiment was performed with cowpea bean seeds, *Vigna unguiculata* cv.
46 Corujinha, between September 2015 and January 2016 at the Chã de Jardim Experimental
47 Farm of the Centro de Ciências Agrárias of the Universidade Federal da Paraíba (CCA-
48 UFPB), in Areia-Paraíba, located in the micro-region of the Paraíba, under the geographic
49 coordinates 6°58'12 "S and 35°42'15" W.

50 According to Graussem's bioclimatic classification, the predominant bioclimate in the area is
51 the sub-dry Northeastern 3dfh with annual rainfall of approximately 1,400 mm. According to
52 Köppen's classification, the climate is characterized as warm and humid, with autumn-winter
53 rains. The average annual temperature ranges from 22 to 26 °C and relative humidity
54 between 75 and 87% [10]. During the conduction of the experiment the minimum
55 temperature was 20.3 °C and the maximum was 28.5 °C, with average relative humidity of
56 76.4%. According to Embrapa [11], the soil of the experimental area is classified as a typical
57 Psamitic Regolithic Neosols, of medium texture.

58 **2.2 Experimental Design**

59 For the soil preparation the area was cleaned with garden hoes and pits at a depth of 4 cm,
60 spaced 0.30 m between plants and 1.0 m between rows were opened. Three seeds/pit were
61 sown, after thinning, one plant/pit was left, the plants were monitored periodically to follow
62 the flowering stage, while the cultural treatments were recommended for the crop.

63 Fifty-four days after sowing, when approximately 70% of the plants started the anthesis they
64 were identified using wool yarns. The plants were monitored until fruiting and, every 3 days
65 were harvested, with a total of seven harvests, manually performed, mechanical injuries in
66 the pods and seeds were **avoided**. After **harvested**, the pods were packed in plastic bags,
67 identified and sent to the laboratory.

68 **2.3 Evaluated Parameters**

69 The pod and seed biometry, and also their physiological quality, were evaluated in the
70 Laboratório de Análise de Sementes, also located in the previously mentioned Center.

71 After each **harvest**, four replicates of 15 pods and 25 seeds were submitted to direct
72 measurements with the aid of a digital caliper, in which measurements of length, width and
73 thickness were performed, the results were expressed in millimeters, only the length of the
74 fruit was expressed in centimeters.

75 After each **harvest**, by using a sample of 40 pods the number of seeds per pod was
76 determined by manual counting, and the results were expressed as number of seeds per
77 pod⁻¹.

78 The water content of the pods and seeds were obtained by the stove method at 105 °C for
79 24 hours [12], using four replicates of 25 seeds and four replicates of 5 pods at each **harvest**
80 period, the results were expressed in percentage.

81 **The samples were placed in a stove at 105 ± 3 °C for 24 hours [12], after that, the dry matter**
82 **of the pods and seeds were determined together with the water content, at all harvest time.**
83 **The results were expressed in grams.**

84 The germination test was performed following the requirements of the Rules for Seed
85 Analysis - RSA [12], using 200 seeds per treatment, distributed in four replicates of 50
86 seeds, placed in a paper towel substrate (**germitest**[®]) moistened with sterilized distilled water
87 in a quantity equivalent to 2.5 times the dry paper weight, distributed on two sheets of paper,
88 covered by a third and organized in the form of rolls, which were packed in transparent
89 plastic bags to avoid the loss of water by evaporation. The rolls were placed in germination
90 chamber of the Biological Oxygen Demand type (B.O.D.) regulated at a constant
91 temperature of 25 °C. The counting was performed five to eight days after the test,
92 considering the normal **seedlings were considered**, characterized by having a long, thin
93 primary root coated with absorbent hairs along the entire surface, well defined lateral roots
94 and well developed shoot, presenting the potential to continue its development and give rise
95 to normal plants, the results were expressed in percentage.

96 The first germination counting was carried out concurrently with the germination test, the
97 germinated **seeds were counted** on the 5th day after sowing [12].

98 For the germination speed index, daily countings were performed, five to eight days after the
99 test, and the index was determined according to the equation proposed by Maguire [13].

100 At the end of the germination test, the normal seedlings of each replicate were measured
101 with a ruler graduated in centimeters, the length of the seedlings were measured, and the
102 results were expressed in centimeters per seedlings. The seedlings previously measured
103 were packed in Kraft paper bags type, taken to a stove regulated at 80 °C for 24 hours and,
104 after that period, weighed in an analytical scale with an accuracy of 0.001 g, the results were
105 expressed in g.plantula⁻¹.

106 **2.4 Data Analysis**

107 The experimental design used in the field was a randomized block, and completely
108 randomized at the laboratory, the results were submitted to analysis of variance and

109 polynomial regression to evaluate the characteristics described previously, the linear and
110 quadratic model were tested, where the significant model of higher order was selected to
111 express the results. The program Sisvar 5.0 was used to perform the statistics analysis at
112 the significance level of 5% probability ($P = .05$). [14].

113 3. RESULTS AND DISCUSSION

114 Changes in the coloration of the pods and seeds were observed during the maturation
115 process (Table 1) and varied from dark green to brown with small dark brown dots.
116 According to Lopez et al. [4], the color of the pods and seeds has been used as a good
117 indicator of the harvest point, however, environmental factors must be observed since the
118 differences in coloring can also be caused by its influence.

119 **Table 1. Color of the pods and seeds of cowpea (*Vigna unguiculata* L. Walp.), cv.**
120 **Corujinha, at different times of harvest.**

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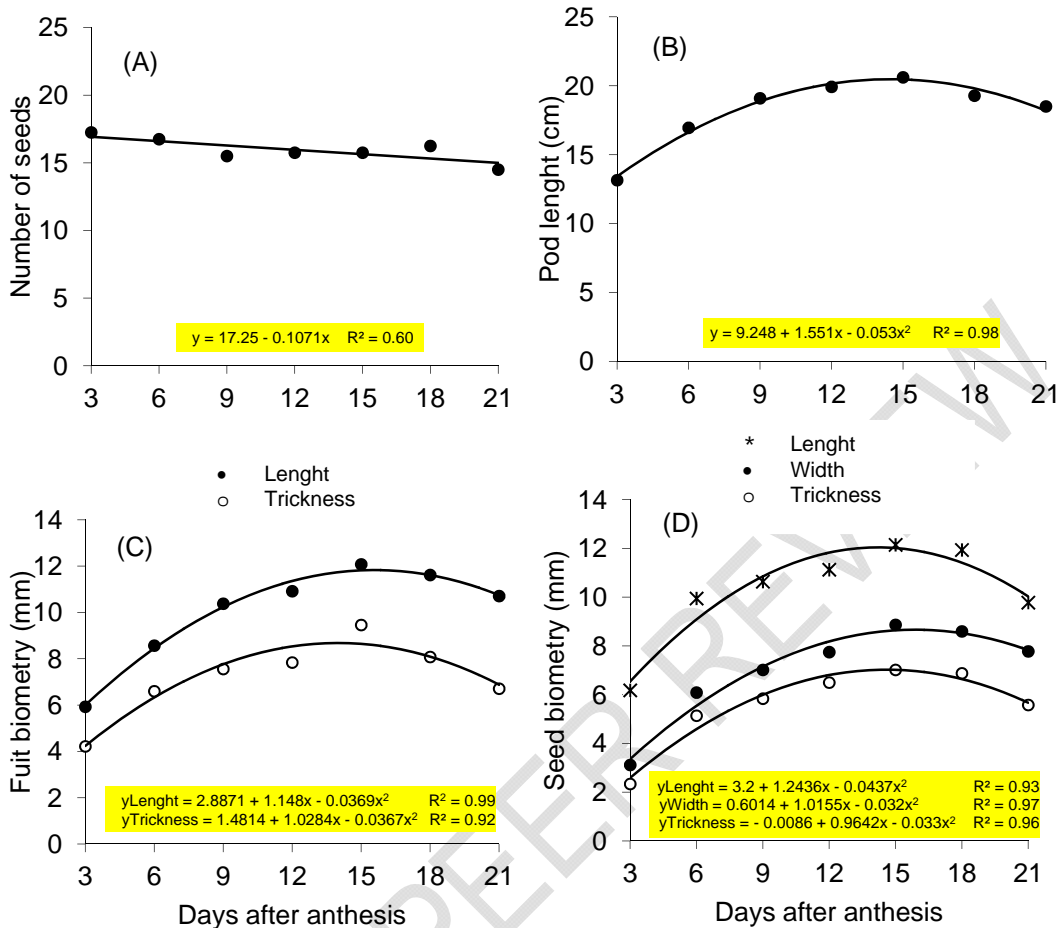
Harvest time	Days after anthesis	Color	
		Pods	Seeds
1 st	3	Dark green	Dark green
2 nd	6	Dark green	Dark green
3 rd	9	Light green	Dark green
4 th	12	Light green	Light green
5 th	15	Light green	Light green
6 th	18	Light brown	Light brown
7 th	21	Brown (dots)	Brown

122

123 For the number of seeds per fruit, a decreasing linear behavior is observed as a function of
124 the harvesting time (Figure 1A) and, in relation to the size of the pods and the seeds, the
125 data were adjusted to the quadratic model, with maximum length of (20.6 cm), width (8.8
126 mm) and thickness (11.8 mm) of the pods obtained at 15 days after anthesis (Figures 1A
127 and B). For the seeds, the maximum length (12 mm), width (8.6 mm) and thickness (7.0 mm)
128 were also verified at 15 days after anthesis (Figure 1D).

129 Similar results were found by Botelho et al. [7] when studying the ideal harvest time for
130 beans (*Phaseolus vulgaris* L.) where was verified a direct relation between seed size and
131 physiological quality, in which seeds of lower size negatively influenced the seed quality of
132 the lot.

133 Padua et al. [15] also verified that larger seeds originated higher soybean plants than plants
134 originated from smaller seeds. According to Carvalho and Nakagawa [16], larger seeds were
135 better nourished during their development, have well-formed embryos and a greater amount
136 of reserves, with greater potential for germination and more vigorous plants when compared
137 to smaller seeds.



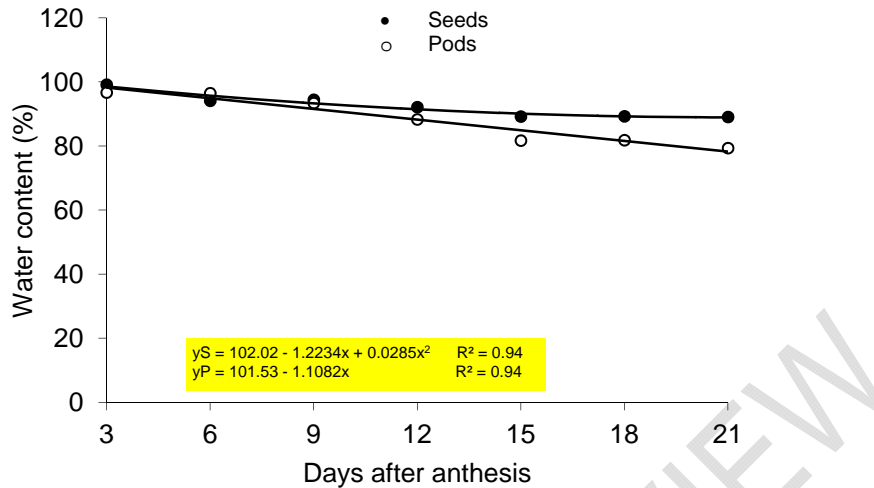
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140 **Figure 1. Number of seeds (A), pod length (B), Fruit biometry (C) and biometry of the**
 141 **seeds (D) of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different**
 142 **times of harvest.**

143 The water content of the pods presented a linear behavior and the seed water content
 144 presented a quadratic behavior according to the harvest times, in which, in the first
 145 harvesting, at three days after the anthesis, the water content was high in the pods (96.7%)
 146 and seeds (99.0%). Then, there was a gradual decrease until the last harvest, 21 days after
 147 anthesis (18% for pods and 10% for seeds) (Figure 2). Botelho et al. [7] found similar results,
 148 and verified a decrease in water content of common bean seeds during the physiological
 149 maturation process.

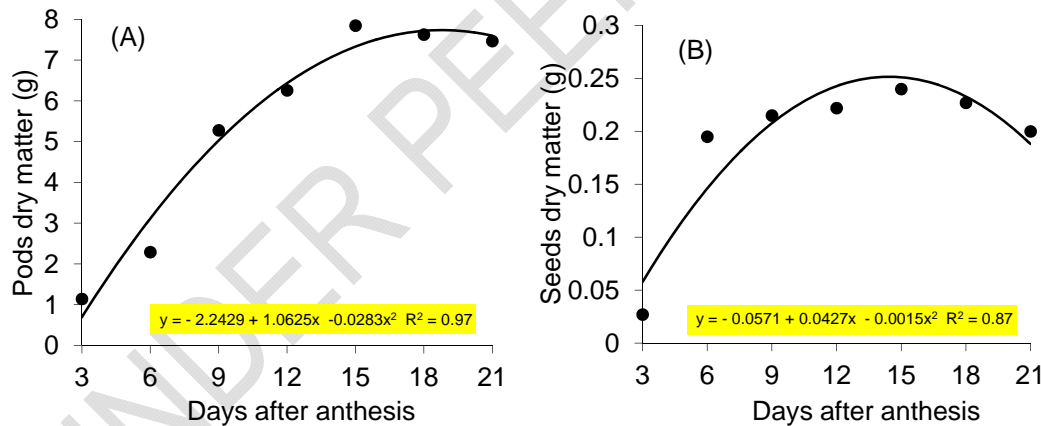
150 However, the water content at the time of harvesting was high and this permanence for a
 151 long period can negatively affect the storage and commercialization of the seeds, which can
 152 result in the reduction of the physiological quality, cause deformations and favor conditions
 153 for the development of fungi, which are factors responsible that accelerate the deterioration
 154 process [3].



155

156 **Figure 2. Water content of the pods and seeds of Cowpea bean (*Vigna unguiculata* L.**
 157 **Walp.), cv Corujinha, at different times of harvest.**

158 For the pods and seeds dry matter, data were adjusted to quadratic models, with maximum
 159 values of 7.85 and 0.240 g, respectively, reached at 15 days after anthesis (Figure 3A and
 160 3B). In the same harvesting time (15 days after anthesis) the maximum dry matter was
 161 observed in the pods and seeds, the water content of the seeds was high, above 80%, and
 162 the germination percentage reached the maximum values.



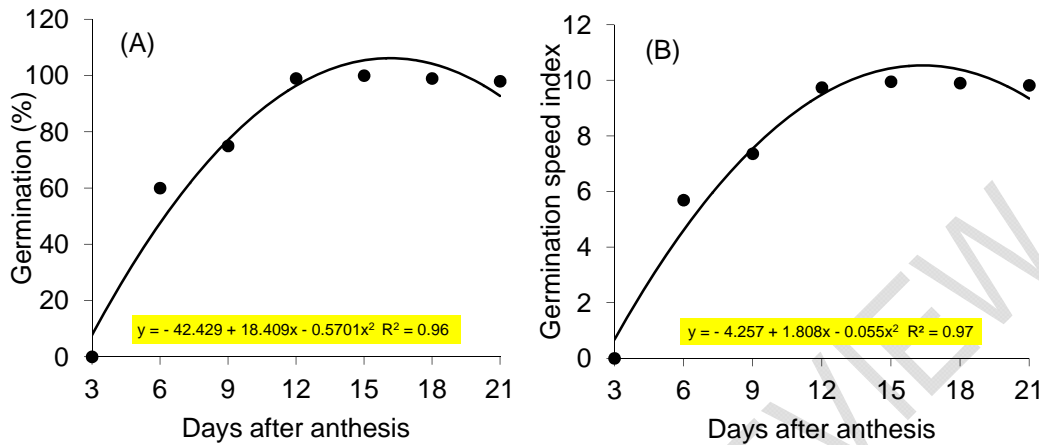
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164 **Figure 3. Dry matter of the pods (A) and seeds (B) of Cowpea bean (*Vigna unguiculata***
 165 **L. Walp.), cv Corujinha, at different times of harvest.**

166 A similar behavior was described by Eskandari [17] in seeds of *Vigna sinensis*, Botelho et al.
 167 [7] and Bolina et al. [18] in common bean seeds and Nogueira et al. [2] in cowpea seeds.

168 The germination percentage and germination speed index were adjusted to the quadratic
 169 model, with the highest values observed at 15 days after anthesis (100% and 9.9,
 170 respectively), remaining high until the last day of evaluation (21 days after anthesis) (Figure
 171 4A and B). Nogueira et al. [2] **evaluated** the development and physiological quality of
 172 cowpea seeds, cv. BRS Guariba, during the maturation process, observed that at 14 days

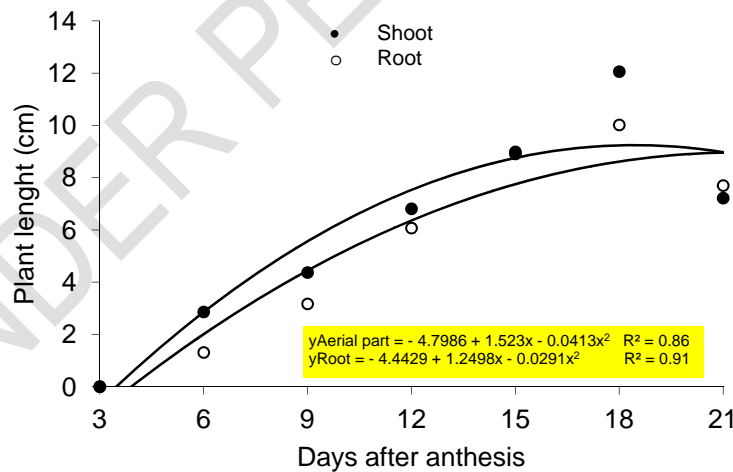
173 after anthesis, the seeds reached their highest percentage of germination and germination
 174 speed index, remaining stable until the last harvest, corroborating with the results obtained in
 175 this work.



176

177 **Figure 4. Germination (A) and germination speed index (B) of seeds of Cowpea bean**
 178 **(*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.**

179 The shoot and root length data (Figure 5) were also adjusted to the quadratic model, where
 180 a gradual increase was observed during the maturation process, with an estimated
 181 maximum value for shoot (12.06 cm) and root (10.02 cm) at 18 days after the anthesis and,
 182 with a subsequent small decrease.



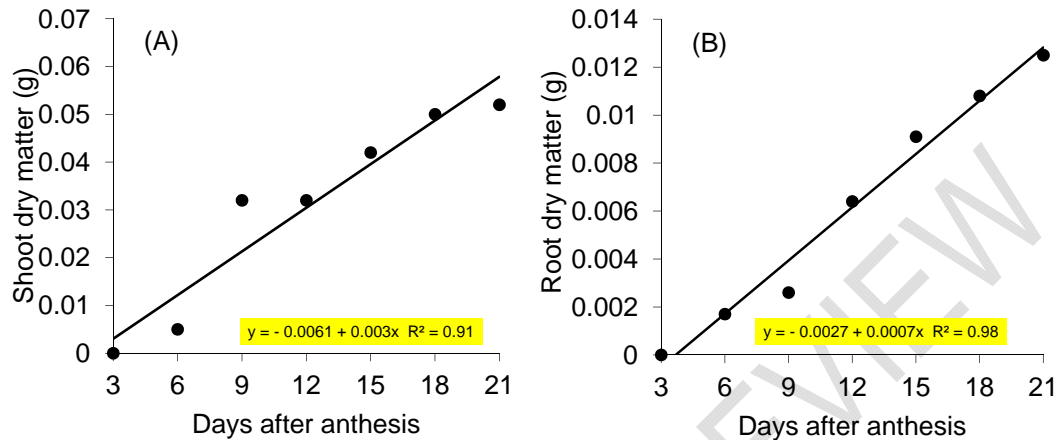
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184 **Figure 5. Shoot and root length of seedlings of Cowpea (*Vigna unguiculata* L. Walp.),**
 185 **cv Corujinha, at different times of harvest.**

186 For the shoot (Figure 6A) and root (Figure 6B) dry matter, a linear and increasing behavior
 187 was observed as a function of the harvest time, reaching its maximum value (0.052 g for
 188 shoot and 0.0125 g for root), at the last harvest, at 21 days after anthesis, which is due to

189 the metabolic and catabolic events of accumulation in the reserves tissue throughout the
190 development of the seed.

191



192

193 **Figure 6. Shoot (A) and root (B) dry matter of seedlings of Cowpea bean (*Vigna***
194 ***unguiculata* L. Walp.), cv Corujinha, at different times of harvest.**

195 A direct relation between the seed size (Figure 1D) and physiological quality results could be
196 verified, where the seeds of higher size were also those with higher percentage of
197 germination (Figure 4A and 4B) and vigor (Figure 5 6A and 6B). According to Carvalho and
198 Nakagawa [16], the size of the seeds may influence the germination and vigor, since larger
199 seeds were well nourished during their development, usually have well-formed embryos,
200 have larger amounts of reserves and are potentially more vigorous.

201 Similar results were also observed by Padua et al. [15], where they evaluated the influence
202 of soybean seed size on the initial growth of plants and their effect on yield, they observed
203 that larger seeds presented higher percentages of germination, vigor and produce plants
204 with higher height at the **harvest** time, with higher yield, when compared to smaller seeds.

205 Therefore, it is important to harvest the seeds when they reach their maximum size,
206 considering that it will result in higher seed quality, uniformity, more vigorous and productive
207 plant stands.

208 **4. CONCLUSION**

209 The physiological maturity of cowpea bean seeds, cv. Corujinha is fast and occurred
210 between 15 and 21 days after the anthesis, the period that correspond to 15 days after the
211 anthesis is the best period for the harvest of this cultivar, which coincides with a greater
212 germination and vigor.

213 **COMPETING INTERESTS**

214 Authors have declared that no competing interests exist.

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