Influence of pretreatments, temperature and the age of the seeds on dodder germination

Tinkoudougou Cathérine SAWADOGO/ ILBOUDO[1*], Djibril YONLI[2], Hamidou TRAORÉ[2] and Joseph Issaka BOUSSIM[3]

Abstract

Dodder (*Cuscuta australis* R. Br.) is an annual obligate stem parasite that have no roots and is totally dependent on its host plant for assimilating nutrients and water supply. *C. australis* infection reduced the total host biomass and the net photosynthetic rates. In order to determine the effect of pretreatments, temperature and age of the seeds on *Cuscuta australis* germination, tests were carried out in the laboratory. Two batches of dodder seeds were used. The batch 1 was harvested during October 2008 and the batch 2 during October 2010, Soaking in water, cooking, scalding and the acid treatment were the pretreatments used between November 2008 and January 2011. Acid-treated seeds were then sowed at temperatures between 5 °C and 40 °C. The results shown that inside each batch, there is a significant difference between the germination rates as a function of pretreatments and the temperature. The higher germination rates were obtained with acid treatment (43% for batch 1; 84.5% for batch 2) and with temperature at 15 °C (59% for batch 1 and 46% for batch 2). This study might serve as a knowledge tool for predicting dodder seeds germination and time of emergence in a perspective of fighting against dodder.

Keywords: Dodder, seeds, germination, temperature, pretreatment.

Influence des prétraitements, de la température et de l'âge des graines sur la germination de la cuscute

Résumé

La cuscute (*Cuscuta australis*) est un parasite annuel obligatoire des tiges qui n'a pas de racines et est totalement dépendant de ses hôtes pour l'assimilation des nutriments et de l'eau. L'infection de *C. australis* reduit la biomasse totale host et les taux photosynthétiques de ces hôtes. Dans le but de déterminer l'effet des prétraitements, de la température et de l'âge des graines sur la germination de *C. australis*, des tests ont été réalisés au laboratoire. Deux lots de graines de la cuscute ont été utilisés. Le lot 1 a été récolté en octobre 2008 et le lot 2 en octobre 2010. Le trempage dans l'eau, la cuisson, l'ébouillantage et le traitement à l'acide ont été les prétraitements utilisés entre novembre 2010 et janvier 2011. Des graines traitées à l'acide ont été ensuite mises à germer à des températures variant entre 5°C et 40°C. Les résultats montrent une différence significative entre les taux de germination en fonction des prétraitements et de la tem-

[1] Institut de Recherche en Sciences Appliquées et Technologies, Département Substances Naturelles, 03 BP 7047 Ouagadougou 03, Burkina Faso.

[2] Institut de l'Environnement et de Recherches Agricoles, 04 BP 8645 Ouagadougou 04, Burkina Faso

[3] Université de Ouagadougou, Unité de formation et de recherche en sciences de la vie et de la terre, BP 03 BP. 7021 Ouagadougou 03, Burkina Faso

[*] Corresponding author, E-mail: icatherine40@yahoo.fr

pérature, à l'intérieur d'un même lot. Les taux de germination les plus élevés ont été obtenus avec le traitement à l'acide (43% pour le lot 1 ; 84,5 % pour le lot 2) et à la température 15°C (59 % pour le lot 1 et 46 % pour le lot 2). Cette étude peut servir d'outil de connaissance des prédictions de la germination et le moment de l'émergence, pour une perspective de lutte contre ce parasite.

Mots-clés : Cuscute, graines, germination, température, prétraitement.

Introduction

The genus Cuscuta includes some 200 plant species that have no roots and parasitize on host stalks (SARIC-KRSMANOVIC and VRBNICANIN, 2015). In some publications more recent, Cuscuta is classified in its own family *Cuscutaceae* (MISHRA, 2009). *C. australi*s is a dodder existing in Burkina Faso. Parasite infection reduced the total host biomass and the net photosynthetic rates, but these deleterious effects decreased with increasing host age (LI *et al*., 2015). Unlike most roots, the dodder root has neither recognizable root cap nor apical meristem (SHERMAN *et al*., 2008) Dodder seeds are physically dormant because of hard seed coats and do not readily germinate without scarification (GHANTOUS and SANDLER, 2012). COLLIN and PELISSOU (1994) estimated the shelf life of dodder seeds in the soil to 10 years or more. Seed dormancy is the main survival strategy of *C. campestris*, allowing persistence in agro-ecosystems (BENVENUTI *et al*., 2005). After germination, the dodder establishes a parasitic contact by wrapping around the leaves and stems of its host. It cuts then any link with the soil and now lives at the expense of these plants. According to LANINI and KOGAN (2005), the attack of the dodder is observed on garlic (*Allium sativum* L.), pepper (*Capsicum annuum* L.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), sweet potato (*Ipomoea batatas* L. (Lam.)) and several *Citrus* species. The economic importance of dodder is due to the fact that it poses a threat to some vegetable crops such as onions, tomatoes, jews mallow and salad rocket and reduces their yield substantially (ZAROUG *et al*., 2014). Dodder, like others pest plants and weeds, constitute a constraint for agriculture and cause significant damage on agricultural production. It is therefore necessary to conduct investigations that would be familiar with these species in order to develop appropriate control strategies to minimize the crop damage. Some methods were developed for managing or preventing dodder infestations in agricultural crop. So, allelopathic impacts of sunflower and caster bean were evaluated by SEYYEDI *et al*. (2013) against *C. campestris* germination properties. (JOHNSON *et al*., , 2016) for example, demonstrated that manipulation of light spectral quality can significantly impact both the ability of parasitic dodder seedlings to locate nearby hosts and their ability to make successful attachments following host contact. But the authors indicated there are potential obstacles to the realization and next steps are necessary for the development and optimization of spectral manipulation methods for use in agro ecosystems.

Studies on the germination of several species of the *Cuscuta genus* were performed. For example, Germination ecology, emergence and host detection in *C. campestris* was carried out by BENVENUTI et al. (2005) and SARIĆ-KRSMANOVIĆ et al. (2013) studied the effects of temperature on germination of *C. campestris*. Most of these studies are located in the temperate climate zone while with climate change, dodder are increasingly observed in the African countries with major attacks. To our knowledge, very few studies have been conducted on the dodder in Burkina Faso. During fields exploration in Ouagadougou (Capital city of Burkina Faso),

ILBOUDO (2011) observed *C. australis* on 42 plant species, including 16 crops and 27 wild plants all contained in 37 genera and 21 families. It is in the context of fight against the parasite that this study aiming to evaluate the effect of temperature, pretreatments and age of seeds, on *C. australis*, germination was undertaken.

Material and methods

Material

The plant material is composed of two batches of seeds of *C. australis*. The batch 1 has been harvested in October 2008 while batch 2 was harvested in October 2010, in vegetable plots in Ouagadougou located between 12° 20'; 12° 25' of North latitude and 1° 27' ; 1° 35' of west longitude All these seeds have been dried under the Sun and kept at ambient temperature until the analysis was carried out in February 2011.

Methods

The evaluation of pretreatments influence on *C. australis* seeds germination.

The trials of the *C. australis* seeds germination have been carried out in the laboratory of the "Centre National de Semences Forestières". The culture medium was composed of Petri dishes containing filter papers and were wet with distilled water. Each dish was covered by its lid in order to maintain internal humidity conducive to germination and kept at ambient laboratory temperature. Four (4) petri dishes were used by pretreatment, each corresponding to a repetition of 50 seeds. The pretreatments in comparison were the following:

Soaking in water (TE).

For each batch of seeds, two soaking times, namely 24 hours (TE 24 h) and 48 hours (TE 48 h), were applied to two different samples. Each time corresponded to a pretreatment.

Scalding (Eb).

This technique consists to soak the seeds in boiled water at 100 °C and to let water cool with the seeds. Two times scalding were also observed on two samples of each batch of seeds: 24 h (Eb + TE 24 h) and 48 hours (Eb + TE 48 h).

Cooking (C)

Cooking consisted to soak the seeds of three samples of each of the two batches of seeds in boiling water. The heating was stopped 1 minute, 5 minutes and 10 minutes after soaking, respectively for each of the three samples. The seeds were left in the same water at the laboratory ambient temperature for 24 h before sowing. There were three pretreatments of cooking which are C 1 mn, C 5 mn and C 10 min.

Treatment with acid (TA)

The seeds of *C. australis* were soaked with agitation in sulphuric acid (H2SO4; 98%). After this soaking, seeds were rinsed thoroughly with water. The seeds are then left in water for 24 h. There were five types of soaking in acid, corresponding to each of a pretreatment: 1 minute = TA 1 mn, 5 minutes = TA 5 mn, 10 minutes = TA 10 mn; 30 minutes = TA 30 mn and 60 minutes = TA 60 mn.

Observations on seedling were made every two days from the day of sowing. The seed is said germinated when the radicle emerges (BOGNOUNOU, 2009). In this study, a seed is considered germinated if its radicle torns the coat and reaches done centimeter long. Germinated seeds were progressively withdrawn from the repetition in order to avoid double counting. Observations were made until the thirty-sixth (36th) day after sowing focusing on:

- **Germination Rate (TG)** which is the proportion of the seeds germinated on a total of 100 seeds; it is calculated according to the formula of International Seeds Testing Association (ISTA, 2007):

TG = n x 100 / N
TG = Germination rate. It is expressed in percentage (%)
n = number of seeds germinated
N = number of seeds to germinate

- **germination latency time** is the time interval between the placing of the seeds to germinate and the first (s) germination (s) (BOUSSIM, 1988).

The evaluation of the temperature effect on the germination of *C. australis* seeds treated with sulphuric acid

Seeds of *C. australis* were firstly treated with sulphuric acid for 30 minutes followed by soaking in water for 24 h (TA 30 mn). This pretreatment was chosen because it gave the highest germination rates during the first tests. The seeds were then germinated in five incubators set respectively at 5 °C, 15 °C, 30 °C, 35 °C and 40 °C.

Statistical analysis of the data.

Data were entered using Excel 2010 and analyzed using the software JMP 9. The germination tests were repeated four times per sample. The results were expressed as mean ± standard deviation. P values <0.05 were considered statistically significant. The comparison of means was made according to the Student's test at the 5% threshold. Germination curves were generated using the Prism 5 software.

Results

Effect of pretreatments on *C. australis* seeds germination

The averages of germination rates of the two batches *C. australis* pretreated seeds, registered with a periodicity of two days until the 36th day, are presented in figures 1 and 2. With the batch 1, the highest germination rates, 38% and 43%, were obtained with respectively TA 30 mn and TA 60 mn. Latency time of germination was two days with TA 5 mn, TA 30 mn and TA 60 mn pretreatments. It has been four days for the control, TE 24 h, TA 1 mn, TA 10 mn, C 1 mn and C 5 mn, Eb + TE 24 h and 6 days for C 10 min, Eb + TE 48 h allowed germination to the 16th day and TE 48 h went to the 30th day. The results shown significant differences between the pretreatments for the batch 1 ($P < 0.0001$).The averages that are not significantly different are followed by the same letter (figure 3).

With batch 2 seeds, highest germination rates were obtained with TA 30 mn, (84.5%), TA 60 mn (79%) and TA 10 mn (74%) (figure 2). Averages with other pretreatments were less than 10%.

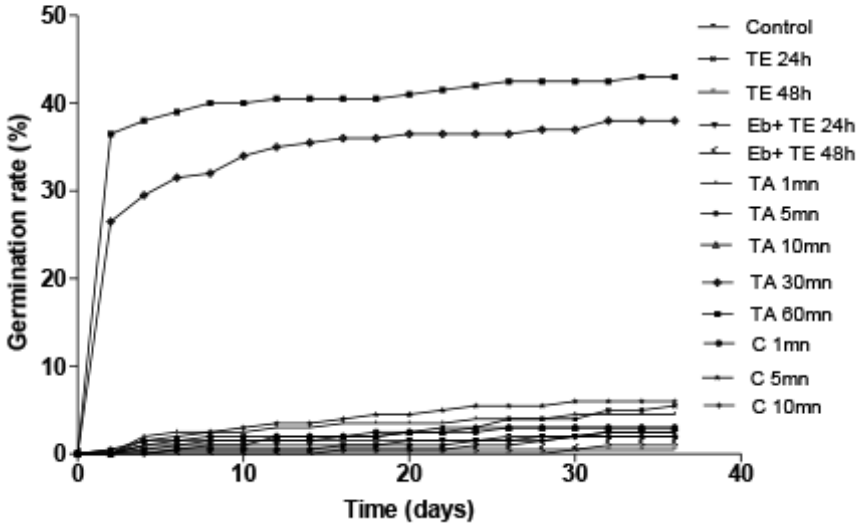

Figure 1. Dynamic of the batch 1 seeds germination of *C. australis* as a function of pretreatments

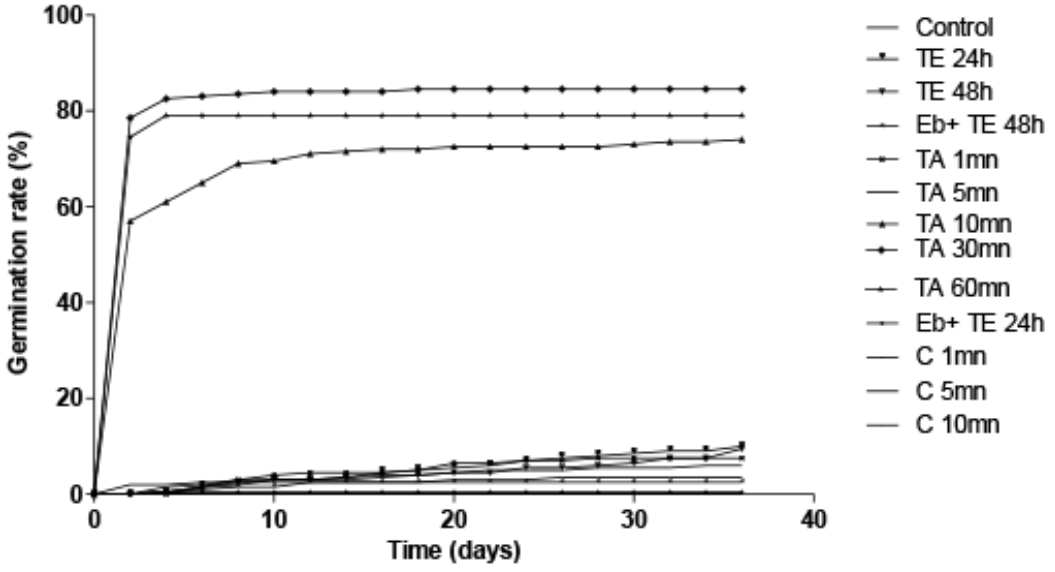

Figure 2. Dynamic of the batch 2 seeds germination of *C. australis* as a function of pretreatments

Latency time was two days for TA 5 mn, TA 10 mn, TA 30 mn and TA 60 mn. For batch 2,latency time was four days with control seeds, for TE 24 h, TE 48 h, Eb + TE 24 h, Eb + TE 48 h and six days with TA 1 mn. The results indicated significant differences between the pretreatments for the batch 2 with $P < 0.0001$ (figure 3).

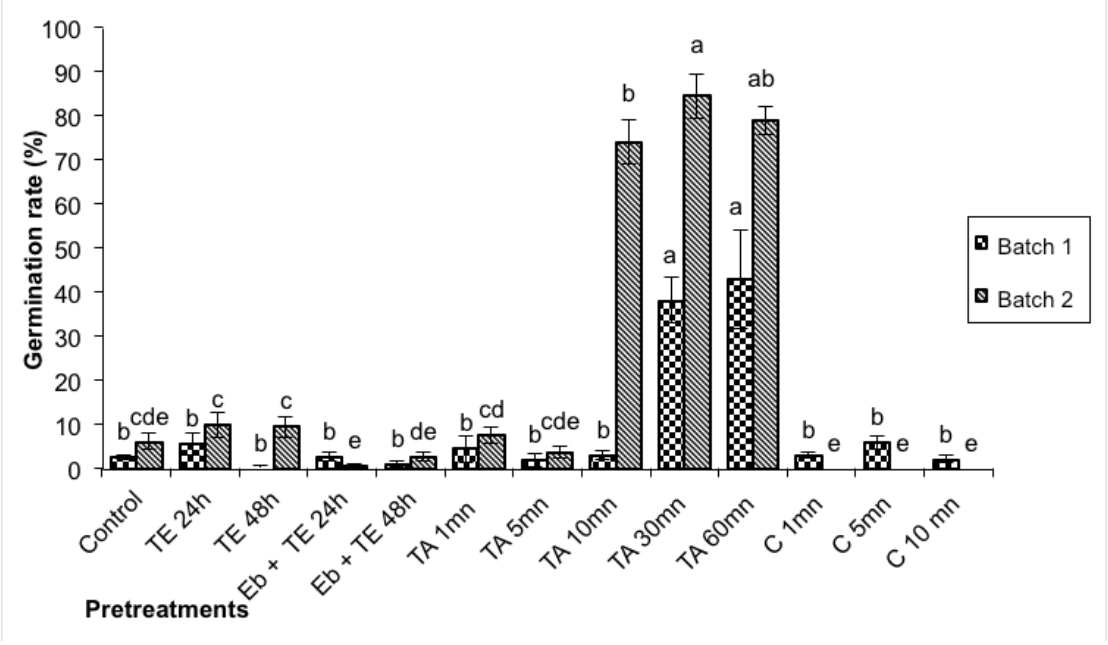

Figure 3. Effects of different pretreatments on germination rate of the two batches of *C. australis* seeds.
In the same batch, the bars followed by a same letter are not significantly different according to the Student's tes

Effect of temperature on the germination of *C. australis* seeds treated with sulphuric acid.

Figures 4 and 5 represent the dynamic of the two batches of seeds treated with acid germination rate depending on the temperature. The highest germination rates of the batch 1 were obtained at 15 °C (46%), 30 °C (42%) and 35 °C (39%). The latency time was two days to 30 °C, 35 °C and 40 °C, four days at 15 °C and twelve days for 5 °C. There are significant differences between the averages of the germination rates of the batch 1 according to the temperature with $P < 0.0001$ (figure 6).

With batch 2, the higher germination rates was found at 15 °C (59%), 30 °C (57.5%), 35°C (52.5%) that are not significant differences between them. However, these three averages are significantly different from the lowest (2% and 35.5%) obtained at 5 °C and 40 °C because $P < 0, 0001$. The latency is two days for 30 °C and 35 °C, four days to 15 °C and 40 °C and twenty-eight days with 5 °C.

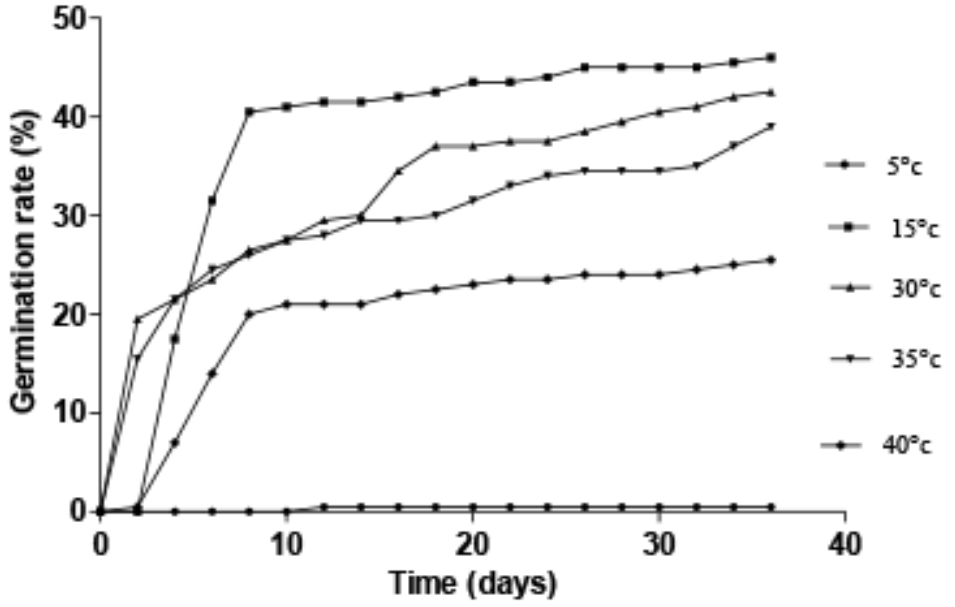

Figure 4. Dynamic of the batch 1 seeds germination of *C. australis* treated with sulphuric acid as a function of temperature.

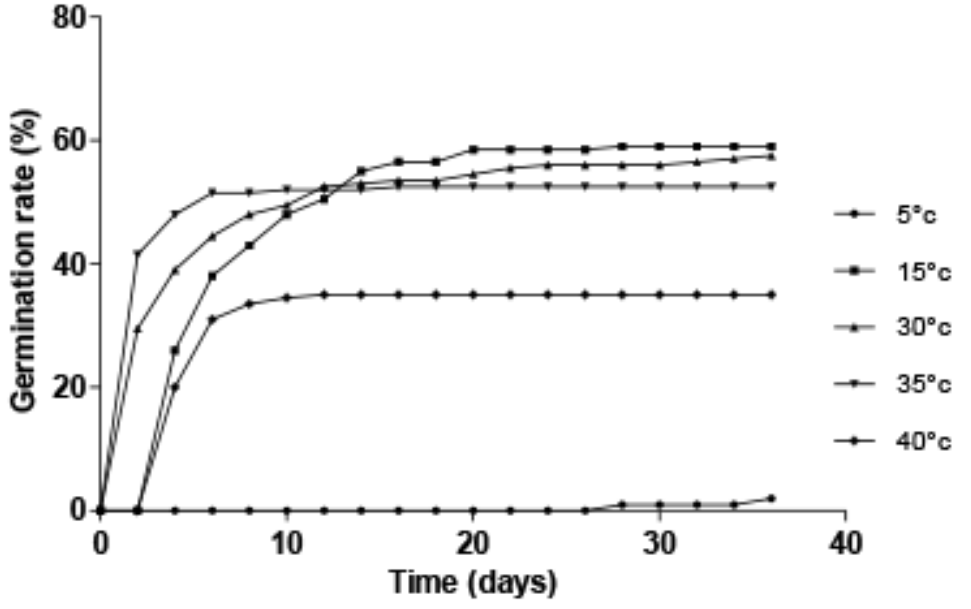

Figure 5. Dynamic of the batch 2 seeds germination of *C. australis* treated with sulphuric acid as a function of temperature.

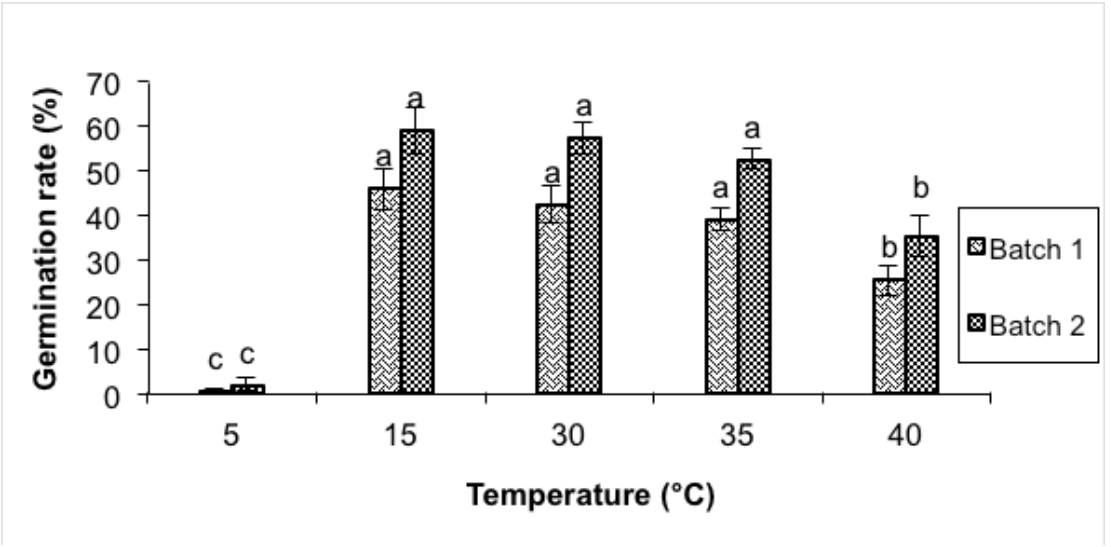

Figure 6. Effects of different temperatures on the germination of the two batches of *C. australis* seeds treated with sulphuric acid.
In the same batch, the bars followed by a same letter are not significantly different according to the Student's test.

Discussion

The highest germination rates of the batch 1 (43%) and the batch 2 (84.5%) were both got respectively with TA 60 mn and TA 30 mn. In addition, most of the seeds treated with the acid between 10 and 60 mn Start germinating two days after sowing. The acid treatment (TA) is therefore the best suited treatment for rapid germination of dodder. This can be explained by the fact that the coats of the seeds soaked in acid for 10 mn, 30 mn and 60 mn were completely eaten by the acid. MISHRA (2009) reported that maximum germination of *C. campestris* and *C. reflexa* was recorded when seeds are treated with acid for a period of 60 min. JOHNSON *et al*. (2016) could also induce germination of *C. campestris* and *C. gronoviivia* scarification with concentrated sulphuric acid for one hour. These seeds shown a whitish appearance at the end of the treatment. This was shown by a study on *C. australis* in Taiwan by JAYASURIYA et al. (2008) where manually scarified seeds germinated to 100 %. Those soaked for 1 min and 5 mn had their coats partially altered and the seeds had a blackish color. Pretreatments based on soaking in water (TE), scalding (Eb) and cooking (C) caused a softening of the coat but did not change its color. This caused a low germination and seed rot. The germination rate of TA 1 mn is higher than those of TA 5 mn and TA 10 mn for the batch 1. These results would be due to the appearance of seeds after pretreatments. TA 1 mn wet seeds without altering their coats. Most of the seeds which have undergone this treatment remained hard at the end of the experiment. However, TA 5 mn and TA 10 mn were partially destroyed coat without detaching it and this caused significant seed rot. Similarly, the other pretreatments with low germination rates (C 1 mn, C 5 mn, C 10 mn, Eb + TE 24 h and Eb+ TE 48 h) caused an important seed rot.

Placed in the same temperature and humidity conditions, non-pretreated seeds have low germination rates compared to those pretreated seeds. This further confirms the tegumentary dormancy

of *C. australis* seeds. This dormancy was reported by many authors on several species of Dodder. DAWSON *et al*. (1994) reported this dormancy on *C. campestris*. COLLIN and PELISSOU (1994) estimated the shelf life of dodder seeds in the soil to 10 years or more.

By comparing the germination rate of seeds treated with acid according to the temperature (figure 6), the results of the batch 2 are similar to those of the batch 1 as highest rates were obtained at 15 °C in both cases. We remarked that between 15 °C and 40 °C, germination rate was decreasing when the temperature was increasing. These results are similar to those of SARIĆ-KRSMANOVIĆ *et al*. (2013) on *C. campestris* seed germination between 5°C and 45°C. They recorded 30ºC as optimal temperature with a germination rate of 96.88%. *C. campestris* seeds best germinated after scarification with sulphuric acid.

The optimal temperature for germination would be between 15 °C and 30 °C. BENVENUTI et al. (2005) found 30°C as the optimal temperature of C. campestris seeds germination.

According to LANINI and KOGAN (2005), the soil temperatures favorable for germination and emergence of Cuscuta spp. are between 15 °C and 38 °C with an optimum around 30 °C. and ITO (1988) recorded the optimal germination of C. japonica at 15 °C with a maximum germination rate of 99%. Germination of fresh *C. campestris* seeds (intact or scarified), as a function of light and temperature conditions (BENVENUTI *et al*., 2005).

At 5 °C, the ungerminated seeds remained intact until the experiment end. Dodder seeds did not germinate at this temperature but remained without any damage. Therefore, *C. australis* seeds could be kept at 5 °C or less. However, at the highest temperature (40 °C), the seeds started to rot from the fourth day of the trial. In addition, seedlings died just two days after germination. A new model describing temperature-dependent *C. campestris* seeds germination has been realized by GOLDWASSER et al. (2016). This study determines temperature germination between 2 °C and 40 °C with an optimal of 32 °C.

The age effecting seeds germination can be deduced from the comparison of the rates obtained with pretreatments (figure 3) and temperatures (figure 6). About the pretreatments, the batch 2 seeds germination rates were significantly higher than those of the batch 1 with control, TE and TA. The batch 2 germination rates were also significantly higher than the rate of the batch 1 at all temperatures. The highest germination rates of the batch 1 (43%) ant of the batch 2 (84.5%) were got with respectively TA 60mn and TA 30mn. The reason could be the low hardness of the coats of the batch 2 seeds which were just harvested compared to the batch 1 harvested 2 years before the trial. The coat hardness would increase *C. australis* seed dormancy but seeds longevity would not reduce. No statistical difference in the germination of intact or scarified *C. campestris* seeds that had been stored for 12 years in laboratory was observed (BENVENUTI *et al*., 2005).

Conclusion

The results shown that *C. australis* seeds dormancy may be removed by seeds pretreating before sowing. Among the pretreatments cooking, soaking in water, scalding, acid treatment used, the one made with sulphuric acid treatment for 30 mn and 60 mn, is the most suitable for dodder good germination. The germination rate of this treatment are significantly upper in relation to the others. Temperature was found to be the important factor of germination of *C. austalis* seeds. The optimal germination temperature was between 15 °C and 30 °C. Age of the seeds had

an influence on germination because of the progressive hardness of the seed coat. This hardness should be the cause of a long viability of these seeds in the soil. Our results contribute to better understanding of *C. australis* germination and emergence, and can be used in developing programs for prevention and control of this pest plant.

Acknowledgments

Our special thanks to:

- CIOSPB (Centre National de l'Information, de l'Orientation Scolaire et Professionnelle, et des Bourses) of Burkina Faso for funding this study through grant.
- CNSF (Centre national de Semences Forestières) of Burkina Faso

We don't forget:

Dr. NARE Alice, Mr. OUATTARA Yacouba, Mr. OUEDRAOGO Abdoul Rasmané, Mr. GARBA Zakaria

They have been of great help in correcting the manuscript of this article.

References

BENVENUTI S., DINELLI G., BONETTI A and CATIZONE P., 2005. Germination ecology, emergence and host detection in *Cuscuta campestris*. Weed Research, 45 (4) :270–278.

BOGNOUNOU F., 2009. Restauration écologique et gradient latitudinal : utilisation, diversité et régénération de cinq espèces de Combretaceae au Burkina Faso. Thèse de Doctorat, Université de Ouagadougou, Burkina Faso, 360 p.

BOUSSIM I. J., 1988. Contribution à l'étude de la Biologie des phanérogames parasites : Recherches sur *Cassytha filiformis L. (Lauraceae*), Mém. DEA, Université Cheick Anta Diop de Dakar, Sénégal, 96 p.

BOUSSIM I. J., 2002. Les phanérogames parasites du Burkina Faso : Inventaire, Ecologie et quelques aspects de leur biologie ; cas particulier des *Loranthaceae* parasites du karité. Thèse de Doctorat d'Etat en Sciences Naturelles, Université de Ouagadougou, Burkina Faso, 306 p.

COLLIN F et PELISSOU J.-L., 1994. Luzerne, Maîtriser la cuscute, c'est possible, Bulletin semences hiver, 126: 25-28.

DAWSON J. H., MUSSELMAN L. J., WOLSWINKEL P. and DÖRR I., 1994. Biology and control of Cuscuta, Rev, Weed Sc., Soc. Am., 6 : 265-317.

GHANTOUS K. M. and SANDLER, H. A., 2012. Mechanical Scarification of Dodder Seeds with a Handheld Rotary Tool. Weed Technology, 26: 485–489.

GOLDWASSER Y., MIRYAMCHIK H., RUBIN B., EIZENBERG H., 2016. Field Dodder (*Cuscuta campestris*)-A New Model Describing Temperature-Dependent Seed Germination. Weed Science, 64 (1) :53–60.

ILBOUDO T., 2011. Biologie et écologie de la cuscute (*Cuscuta australis* R. Br.), parasite de certaines plantes cultivées au Burkina Faso. Mémoire de DEA, Université de Ouagadougou, Burkina Faso, 65p

JAYASURIYA K. M. G. G., BASKIN J. M.., GENEVE R. L., BASKIN C.C. and CHIEN C.-T., 2008. Physical dormancy in seeds of the holoparasitic angiosperm *Cuscuta australis* (*Convolvulaceae, Cuscuteae*): Dormancy-breaking requirements, anatomy of the water gap and sensitivity cycling. Annals of Botany, 102 (1) :39–48.

JOHNSON B. I., DE MORAES C. M. and MESCHER M. C., 2016. Manipulation of light spectral quality disrupts host location and attachment by parasitic plants in the genus Cuscuta. Journal of Applied Ecology, 53 (3) : 794–803.

LI D., WANG L., YANG X., ZHANG G. and CHEN L., 2010. Proteomic analysis of blue light-induced twining response in *Cuscuta australis*, Plant Mol .Biol., 72 : 205–213.

LI J., YANG B., ZHANG J., YAN M., & LI M., 2015. Effects of a native parasitic plant on an exotic invader decrease with increasing host age. AoB PLANTS Advance Access: 1–31.

LANINI W. T. and KOGAN M., 2005. Biology and Management of Cuscuta in Crop, Ciencia e Investigacion Agraria, 3 (32): 165-179.

MISHRA J. S., 2009. Biology and Management of *Cuscuta* species. Indian J. Weed Sci., 41 (1&2) : 1–11.

ISTA (Règles Internationales pour les Essais de Semences), 1999. ISBN 3-906549-27-5, 362 p.

SARIC-KRSMANOVIC M., BOZIC D.,PAVLOVIC D., RADIVOJEVIC L. and VRBNICANIN S., 2013. Temperature Effects on *Cuscuta campestris* Yunk . Seed Germination. Pestic. Phytomed. (Belgrade), 28 (3) :.187–193.

SARIC-KRSMANOVIC M. and VRBNICANIN S., 2015. Field dodder - how to control it? Pestic. Phytomed. (Belgrade), 30(3): 137–145.

SEYYEDI M., MOGHADDAM P. R., SHAHRIARI R., AZAD M. and REZAEI E. E., 2013. Allelopathic potential of sunflower and caster bean on germination properties of dodder (*Cuscuta compestris*). *African Journal of Agricultural Research*, 8(7): 601–607.

SHERMAN T. D., BOWLING A. J. C., BARGER T. W., and VAUGHN K., 2008. The Vestigial Root of Dodder (*Cuscuta pentagona*) Seedlings. International Journal of Plant Sciences, 169 (8): 998–1012.

ZAMBETTAKIS C. and MAGNANON S., 2008. Identification des plantes invasives de Basse-Normandie, Conservatoire Botanique National de Brest, 28 p.

ZAROUG M. S., ZAHRAN E. A. B., ABBASHER A. A. and ALIEM E. A. A., 2014. Post-attachment herbicide treatment for controlling Field Dodder (*Cuscuta Campestris* Yuncker) parasitizing onion in Gezira State ,. Int. J. Pharma Bio Sci., 3(5) : 123–127.