The evolution of non-structural carbohydrates in the wild jujube "*Ziziphus lotus* (L.) Desf. " and chemical control strategy

RSAISSI NOUREDDINE
Department of health and agro food
Faculty of Sciences and Techniques
Settat, Morocco

BOUHACHE MOHAMED
Department of Production, Protection and Plant Biotechnologies
Institute of Agronomy and Veterinary Hassan II
Rabat, Morocco

BENCHARKI BOUCHAIB
Department of health and agro food
Faculty of Sciences and Techniques
Settat, Morocco

Abstract—**The evolution of non-structural carbohydrates (TNC) in the wild jujube "*Ziziphus lotus* (L.) Desf." was determined monthly during one year in the region of Chaouia, Morocco. The TNC contents of roots and stems vary in time depending on the phenological stage of the plant. In the beginning of the development cycle of this shrub, when vegetative recovery occurs in February, the TNC reserves began to decline smoothly till it reached their lowest level in full vegetative growth period (March and April). Moreover, recovery of these reserves began from the beginning of flowering stage (May-June). After that, the TNC content reached its maximum level at the end of August, which coincides with fruit maturity. The final TNC reserves in the root system at the end of physiological and phenological cycle of the shrub were equal to the ones determined during the dormant stage. The implications of these results for reasoning strategy of chemical control against the wild jujube in fields where this shrub behaves as a weed were also discussed and confirmed by field trials. The results of these tests showed that the highest efficacy was obtained on clumps treated with glyphosate (720g/hl) at early fruit formation, or green drupe fruit maturity, coinciding with the period of recovery TNC reserves.**

Keywords- Contents of TNC, Ziziphus lotus (L.) Desf, phenological stage, chemical control.

I INTRODUCTION

In Morocco, the wild jujube (*Ziziphus lotus* (L.) Desf.) Is known as “sedra” and is present in several biotopes of arid and semi-arid regions. It grows on all soil types [1]. This shrub also behaves as a weed in several crops, including winter and spring cereals, food legumes and orchards. It occurs in several regions of Morocco such as Chaouia [2, 3], Haouz, Zear, Rhamna, the Middle Atlas [4], Gharb [5], the region of Errachidia [6], the Souss [7], the coastal region of Safi, Sidi Ifni region, Khenifra, eastern Morocco, the Sahara and in the region of Oujda [8]. Recently, this shrub is expanding over thousands of hectares of Chaouia region agricultural land where it is a handicap for farming activities in reducing agricultural area and poses serious problems, resulting often heavy production losses [3]. Moreover, several eradication actions of this species, based on glyphosate herbicide, have been launched by the Provincial Direction of Agriculture in the affected areas of Chaouia region. However, the complete chemical control of this shrub was a very difficult task with this herbicide. Indeed, the maximum effect is achieved if the herbicide is applied in optimal conditions and at susceptible growth stage. During the development cycle of a perennial weed, there are specific physiological stages where the effectiveness of a chemical or mechanical treatment is at the maximum. Carbohydrates are translocated from source (mainly leaves) to sinks (the rapidly growing or storage organs) in perennial plants. As the plant grows, the sink source relationships are constantly changing. The study of the evolution of carbohydrates (TNC) is used as good indicator or/and to detect these phenological growth stages [9]. Accurate data dealing with TNC evolution in wild jujube is absent in Morocco. To set up or implement any strategy to control this perennial species in agricultural lands, the stakeholders need to know when this shrub is susceptible to phloem-mobile herbicides. The first objective of this study is to investigate the evolution of non-structural carbohydrates in wild jujube "Ziziphus lotus (L.) Desf." Roots and stem. The second objective is to assess and relate the efficacy of glyphosate to growth stages of the species.

II MATERIAL AND METHODS:

A. *Study site:*

The study was carried out in the Chaouia region of Morocco and precisely in the center area of the province of Settat. This region has a semi-arid climate with an average of annual rainfall of 331 mm and intra-and inter-annual variations: one year on three years is dry. Seasonal variations in monthly temperatures show peak temperatures in summer (July-August), which can reach 39.9 ° C and low temperature peaks during the winter (January-February) to 3 ° C. Thermal amplitudes are relatively high and range from 12 in winter to 20.5 ° C in summer.

B Study of non-structural carbohydrates (TNC) evolution:

1. Sampling and preparation of samples:

Four samples of each part (roots and stems) were collected every month, during a year, from wild jujube patches (5 m² of surface) in different places. Each sample collected was separated into two parts roots and stems. Each part was kept in plastic bags and immediately placed in a freezer. After that, it was brought to the laboratory and the samples were dried in an oven at 100 °C for one hour to stop respiration [10]. Then, they were subjected to dry at 80 °C for 48 h. Once dried, they were ground using an electric grinder with a sieve with a mesh of 0,425 mm (40 mesh). Ground samples were stored in glass vials closed and returned back to the oven at 100 °C for 30 min to complete drying. At the end of drying and after cooling, the vials were then tightly closed and kept for the analysis of non-structural carbohydrates.

2. Extraction of non-structural carbohydrates:

Samples of 0.5 g of each ground sample were used for the acid extraction of TNC. For this, we adopted the method of Smith [10] slightly modified by using 0.2 N hydrochloric acid instead of sulfuric acid 0.2 N in accordance with Bouhache's work et al [9]. Thus, we proceeded as follows:
1. Attack or acid hydrolysis, in flasks of 500 ml ground-necked and refrigerant pipes connected to reflux: we have 0.5 g of plant powder and 60 ml of 0.2 N HCl;
2. Boiling the balloons on a hot plate for two hours, stirring is performed 2 to 3 times during the hydrolysis;
3. Filtering solutions in flasks with a capacity of 100 ml;
4. Adding distilled water to the filtrate to 100 ml.
5. Homogenization of the final solution (filtrate) by stirring.

3. Staining and reading spectrophotometer:

Staining technique we used is the method of Anthrone [11, 12]. To prepare the reagent, we dilute 330 ml of pure sulfuric acid (H_2SO_4) in 140 ml of distilled water. When the mixture has reached room temperature, we added 5 g of thiourea and 0.25 g Anthrone then we stirred with magnetic stirrer. R reagent thus obtained was kept four hours in the refrigerator before use.
The filtrates obtained after acid extraction were diluted to 1/5. An aliquot of 1 ml which was taken from each dilution was added to 10 ml of reagent R in tubes. The tubes were placed in a water bath at 100°C for 15 min. Then they were cooled under running water. After cooling, we immediately read the optical density using a spectrophotometer at a wavelength of 620 mm. For each sample, we have provided two tubes in duplicate, in order to remove the tubes showing any difference that exceeds 10%. A standard solution of glucose of 0.1 mg.ml^{-1} was used as a standard solution.
The concentration TCN is calculated using the following formula [9]:

TNC (%) = (AE ÷ AGS) × 10 With: AE: Absorbance of sample; AGS: Absorbance of standard solution of glucose.

C Field chemical control trial:

The trial dealing with chemical control of the wild jujube was conducted on a fallow and focused on the study of the effect of growth stages application on the efficacy of glyphosate. Thus, a rate of 720g/hl of herbicide was applied to clumps of wild jujube at four different stages (early fruit formation, green drupe, fruit maturity and end-mature- fruit drop). The herbicide was applied with a backpack sprayer equipped with a mirror nozzle. The experimental layout used was a complete randomized block design with three replications. Due to the weed is distributed as patches; we have taken wild jujube clump as the experimental unit .

D Statistical Analysis

The results obtained were subjected to analysis of variance. Multiple comparisons of means were performed with suckled LSD test at a probability level of 5% using the Statistix software.

III. RESULTS AND DISCUSSION:

A Evolution of non-structural carbohydrates

The analysis of the trends of non-structural carbohydrates (TNC) in wild jujube (Ziziphus Lotus (L.) Desf) shows that roots and stems functioned as storage organs. The levels of these carbohydrates in stems and roots vary with time (Fig. 1).

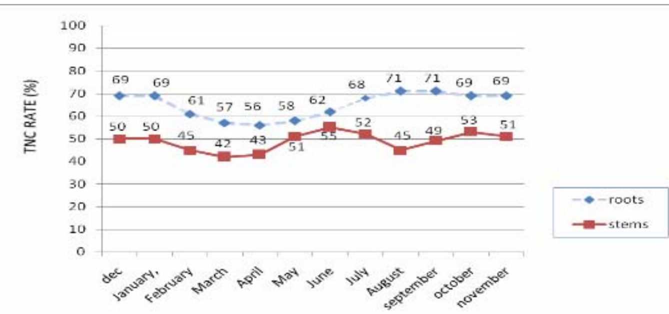

Figure 1 : the evolution of non-structural carbohydrates (TNC) in wild jujube (Ziziphus Lotus (L.) Desf)

Carbohydrate reserves in the roots are more important than those of stems. In general, the highest TNC concentrations were obtained during the period of July to January and the lowest levels were noted during February to June. During vegetative recovery in February, a decreasing in the carbohydrate reserves was observed in different parts of the shrub. At that time the rate of TNC was about 61 and 45%, respectively for roots and stems. This rate continues to decrease until its lowest level (56 and 43%) in March and April, coinciding with full vegetative development. Starting from flowering stage (May-June), the replenishment of root TNC parties began to reach their maximum level (71%) at the end of August, which coincides with the maturity of the fruit. The final TNC reserves (69%) in the root system at the end of physiological and phenological cycle of the shrub were equal to those determined in dormant stage. In general, most perennial plants accumulate TNC during periods when elaboration of carbohydrates exceeds the demand for respiration and formation of new tissues. The obtained results agreed with this general scheme.

These results highlighted the importance of the root system as storage organ of non-structural carbohydrates in wild jujube. However, the stems have stored significant amounts of TNC. The presence of these carbohydrate reserves shows high ability of the species to reproduce and regenerate.

The Glyphosate herbicide, a foliar systemic phloem mobile, should have a good efficiency if its application is made post-flowering stages which coincide with translocation of carbohydrates to the roots. However, this efficiency can reach its maximum if the application of glyphosate is done in stages where migration of carbohydrates to the root system is fast.

B Effect of treatment stages on the efficacy *of glyphosate*

The field trial results presented in Table 1 have confirmed the relationship between the stage of implementation and efficacy of a herbicide treatment. Indeed, the highest efficacy and the most complete control were obtained when wild jujube clumps were treated with glyphosate (720g/hl) at the beginning of fruit formation, or green drupe- fruit maturity, coinciding with the period of reconstitution of TNC reserves in the wild jujube.

TABLE 1, EFFECT OF TREATMENT STAGE OF WILD JUJUBE ON THE EFFICACY OF GLYPHOSATE

Stage of application	**efficacy in%**		
	30 DAT	***60 DAT***	***VR***
Beginning of fruit formation	90 b	100	100 a
green drupe	90 b	100	100 a
Fruit maturity	100 a	100	100 a
End maturing -fruit drop	100 a	NE	90 b

DAT: days after treatment; VD: vegetative restarting the year following treatment; NE: not evaluated (period falling leaves). Numbers followed by the same letter, within the same column, do not differ significantly according to the LSD test at 5%.

The maximum effect of the glyphosate is obtained when wild jujube completes its vegetative growth stage and enters to the reproductive phase and production. Indeed, at this stage, the shrub begins to accumulate its reserves in fruits, roots and stems. As a phloem mobile herbicide, glyphosate follows the movement of carbohydrates in the plant. Thus, the herbicide moves from the site of production of sugars that are leaves to sites use (wells). This source sink relationship vary according to the phenological stage of the plant. These reserves provide energy to méristimatic cells used to the genesis of buds the following year. Carbohydrates (products of photosynthesis) by glycolysis reaction between the acid formation cycle organic acids to give then amino acids are used for protein synthesis. The phytotoxic action of glyphosate is the blockage of synthesis of these amino acids by inhibiting the enzyme EPSP (5-enopyruvyshikamate-3-phosphate synthase) [13]. To promote this reaction and achieve the overall efficacy of glyphosate, it must be applied to fragile stage, when the plant provides an important effort to replenish its metabolic reserves and migration of carbohydrates to the root system is fast. This principle for the destruction of perennials plants has been confirmed by the work of several researchers [9, 14, 15]

IV. CONCLUSION:

The results of this study shows clearly that the strategy of the jujube chemical control by using glyphosate and based on the evolution of non-structural carbohydrates (TNC) in this plant, is a promising way for the development of agricultural lands where this shrub behaves as a weed. Susceptible growth stages that coincide with the intense migration of carbohydrate reserves from leaf (organs of production) to sites of utilization (storage organs: roots) are early fruit formation, or green drupe fruit maturity.

REFERENCES

[1] T. INESCO et SAUVAGE, “Fichier des espèces climax,” Al Awamia, 16, 17-21, 1965.
[2] A. TALEB, C. BOULET et A. CHETIOU, “Etude phytoécologique des adventices des céréales dans la Chaouia,” Acts. Inst. Agrn. Vet. , vol 9(3 et 4), 35 – 43, 1989.
[3] N. RSAISSI, M. BOUHACHE et B. BENCHARKI, “ Importance et Impact agro-économique du jujubier (Ziziphus lotus) dans la Chaouia,” Revue Marocaine de Protection des Plantes 3, 13-27, 2012.
[4] M. TAHRI, “ Etude floristico - Agronomique des adventices vivaces des principales cultures des différentes régions agricoles du Maroc Central et occidental,” Mémoire pour l'accès au grade d'Ingénieur en Chef. MARA, Rabat, 1997.
[5] A. TALEB, “Flore lustre des principales mauvaises herbes des cultures du Gharb (Maroc), These de doctorat Esc Sciences Agronomiques, IAV Hassan II, 309 p+ annexes, 1995.
[6] H. GOUIAA, “Diversité floristique de la zone aride de la province d'Errachidia,” Mémoire d'Ing. d'Etat en Agronomie, I A V Hassan II, Rabat, 1998.
[7] C. BOULET, M. BOUHACHE, M. WAHBI et A.TALEB,“Les mauvaises herbes du Souss,” Documents scientifiques et techniques, 295 p, 1991.
[8] A.C. SANAD, “Contribution à l'étude des conditions écologiques de développement du genre Ziziphus Mill. dans son aire naturelle d'extension et les caractéristiques d'installation de Z. Spina - Christ (L.) Wild (Imbibition, dormance, germination) ,” Mémoires de 3ème cycle pour l'obtention du diplôme d'Ingénieur d'Etat, I A V Hassan II, Rabat, 1999.
[9] M. BOUACHE., C.BOULET et F. EL KARAKHI, “Evolution des hydrates de carbone non structuraux chez la morelle jaune (Solanum elaeagnifolium cav.)”, Weed Research, 33, 291-298, 1993.
[10] D. SMITH, GM. PAULSEN et CA. RAGUSE, “Extraction of total avaible carbohydrates from grass and legume tissue, Plant Physiolgy, 39, 290-292, 1974.
[11] RP. MURPHY, “Extraction of plant samples and the determination of total soluble carbohydrate,” Journal of the Sciences of Food and Agriculture 9: 714-717, 1958.

[12] JH. ROE , "Determination of neutral sugars," Journal of Biological Chemistry, 212, 335, 1955.

[13] R. SCALLA et Ch. GAUVRIT, "Mécanismes d'action phytotoxique des autres familles d'herbicides," In : les herbicides modes d'action et principes d'utilisation, Edi INRA, Paris, 115-191, 1991.

[14] PA. BANK, MA. KIRBY et MAN Pw. SANTEL, "influence of Post emergence and subsurface layered herbicides ou horseneele and peants," Weed science, 25, 5 – 8, 1977.

[15] F. SEVERIN et TISSENT, "Principes de l'utilisation des herbicides," In : les herbicides mode d'action et principes d'utilisation, Edi INRA, Paris, 281-332,1991.