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Investigation of the physiological changes in (*Rosa canina* L.) plant members comprising and not creating the gall

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Abstract. Chlorophyll a, Chlorophyll b, Carotenoid, total chloropyhll concentrations, Chl-a/Chl-b ratio, Total Chlorophyll/Carotenoid ratio, sugar and starch and total carbohydrate amounts, dry biomass changes and proline and sugar amounts interaction at fruit galls of Diplolepis mayri Schld. On *Rosa canina* L. were studied. We found that Chl-a, Chl-b, carotenoid concentrations increased but Chla/Chl-b ratio decreased and total Chlorophyll/carotenoid ratio did not change with gall occurunce in leaves of galled plants. Sugar content at leaves of galled plants were found to be higher than sugar content of control group with galls. In contrast, starch content decreased. As total carbohydrate amount of galled plants declined with gall occurunce but increased with gall maturation. Generally, we found that dry biomass change and sugar content changes of leaves were similar. Monthly changes of sugar levels in fruits decreased depending on the nutrition of larva in galled fruits. It was also found that there is a similarity between the changes of proline and sugar levels as galls development. Proline levels were found to be very high in galled fruits of plants studied.

Key Words: *Rosa canina*, gall, total chlorophyll, total carbonhydrate, dry biomass, proline.

1. INTRODUCTION

As a latin name Gall word is taken from the word of 'Galla' and described as abnormal growth from of plant for defense reaction against parasites, insects, nematodes, mites, bacteria or fungi live in symbiosis on the plant that cause irritation and nutritional physiology of plants. However, the most common cause of galls is a group of insects formed afid, phylloxerans, psyllids, trembling flies and cynipid bees (gall bee) Cynipid bees (Hymenoptera: Cynipidae: Cynipini), located approximately in the 1300 described species (Liljeblad the most complex, most of the best-organized galls are fotmed by gall bees [1] Most well-known cynipid galls present on rose and oak [2].

Cynipid bees also have been found to control on the physiology of gall tissues [3-5]. According to studies, gall tissue provides physiological source flux for nutrients and assimilation products, addition to this, it is also provides source for tannin and phenolic compounds which are intensive especially in external parenchyma cells (they are not included at

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internal tissues of gall) and serve as feeding inhibitors for herbivor insects [3, 6]. Plant galls are complex formation evolving under the influence of two organisms both forming host plant and gall. Abnormal differences in plant cells induced by gall-makers and the response of containing hypertrophy (a noticeable improvement in the volume of cells) resulted in a characteristic growth in some tissues of the host plant to be used as a shelter of gall maker [7]. There is opinion that relationship of some gall makers-host plant is not adaptive or mutualistik [8].

According to the opinion of some plant-based, the formation of gall is perceived as a defense mechanism [7]. Another hypothesis is 'nutrition hypothesis' [9-11], in which it is claimed that not only galls could start to occur for the defense of plants, but also to increase appropriate conditions for themselves, insects stimulate the growth of gall. Galls of plant reduce the amount of nutrients [9-12] and gall-makers use the concentration of nutrients and other components to meet the needs of the developing larvae by increasing dietary substrates, [11, 13, 14]. Therefore, the formation of a gall is a form of adaptation. Galls originally green in color, while the development progresses, the surfaces especially the gaining sun turns into red, in autumn dry and turn into brown. Forming gall rate of Rosehip plants increases up to 90%. Because of the fact that damaged fruits were converted to gall form, they become completely unusable [15]. Studies in our country related Rosehip pests and their natural enemies are very limited. However, in general, some members of the groups of Acarina, Homoptera, Coleoptera and Lepidoptera are defined as a pest of Rosa sp. It was especially determined that Cephidae, Argidae, Tenthredinidae families of Hymenoptera team have the most damaging effect on this plant. On the other hand, ıt was found that insects in the family Cynipidae of Hymenoptera team (*Diplolepis mayri,* etc.). caused the intense formation of fruit gall in Rosehip plant and these galls had negative influence on plant growth and development as well as in fruit yield [15].

In this study, in the rose hip (*Rosa canina* L.), which has lots of important using field, it was aimed to determine changes of carbohydrates, pigments, proline playing an important role on especially gall-plant interaction, also evaluate the relationship between the physiological and biochemical factors.

2. MATERIALS and METHODS

Collection of samples used in the analysis

Samples used in the analysis have been taken Karaçayır from 25 km Sivas. The first, gall samples were collected in the region in April of 2005 and this gall samples placed plastic

cups their mouths closed in cheesecloth cloth, and stored at 21 ^{o}C in a climate chamber. Controls of these samples were done every day, insect species collected and sort of insect species were identified in the entomology laboratory. Out of insects from galls continued about three months. As a result of insect species diagnosed, it was found that *Diplolepis mayri* was responsible for forming of gall. In May and June of 2005 continuous controls are made in this region, in order to the correct diagnosis of plant species, plant groups kept under observation including the flowering time.

Selected plant groups marked with different color ribbons and in June leaves, during July and August the leaf and fruit samples were collected between the hours of 13:30 to 14:00. 10 with and 10 without galls collected from rose hips plant materials were used in the analysis.

These plants collected from in the region of Karaçayır as the starting point of 1731 m. height and N 39^{o} 55 '56.7" EO 36° 59' 35.8"coordinates, as the last point of 1728 m. height and N 39^{o} 55 '45.2 "EO 36 ° 59' 18.3"coordinates.

Analysis procedure

Extraction and purification of pigments

Chlorophyll-a (Chl-a), Chlorophyll-b (Chl-b) and carotenoid extraction, purification and determination

1 g of fresh shoot ground samples was made into 50 ml of acetone (99.5%). Samples were homogenized for 10 min in acetone. Homogenized samples in which by adding 1 / 5 distilled water, were homogenized again. Homogenized samples were centrifuged 10 min. at 3000 cycle / min. Absorbance values of centrifuged samples were read at 662, 645 and 470 nm , and the amounts were identified [16].

Chl-a = $11.75.A_{662}$-$2.35.A_{645}$

Chl-b = $18.61. A_{645}$-3, $96.A_{662}$

Carotenoids = $1000A_{470}$-2.27 Chl-a-81.4 Chl-b / 227

the absorbance coefficient for Chl-a =11.75

the absorbance coefficient for Chl-b =18.61

2.27 Chl-a and 81.4 Chl-b = the absorbance coefficient for carotenoids coefficient

A = absorbance value of (16).

Measurement of total sugar level

0.1 g sample was taken dried and powdered samples, then a few drops of ethyl alcohol (80%) poured on this sample. By adding 5 ml of water mixture was stirred and 25 ml of 80% ethyl alcohol was added and stirred again. After that mixture was centrifuged at 5000 rpm / min for 20 min then taken in a tube. Again by adding 30 ml of 80% ethyl alcohol was stirred and centrifuged. The color was removed by using activated charcoal, it was filtered with Whatman No 42 paper and the volume was completed 100ml with distilled water [17]. The experiments were performed in three replicates. For the determination of total sugar in the extract 1 ml was taken for test tubes and 10 ml 0.15% anthron solution was poured on mixture. After putting anthron in all the series, series were left at 95 º C for 8 minutes. Then they were taken immediately in ice bath to reach room temperature. So, results were read in Spectrophotometer at 625 nm against glucose standards. The total amount of sugar in samples was determined by using of curves prepared according to the standard solution absorbance [18].

Extraction and determination of starch

After analysis sugar, 5 ml of cold water and 6.5 ml of 52% perchloric acid added on the residual, the mixture was shaken for 15 min. Then 20 ml of water was added, mixed and centrifuged. The samples were taken in a centrifuge tube. Again by adding 5 ml cold water and 6.5 ml of perchloric acid on the residue, mixture was shaken for 15 minutes and centrifuged again and waited 30 minutes at 0^{o}C. Solutions combined, filtered and completed to 100 ml [17]. The experiments were performed in three replicates. In order to determination of starch, the test tubes were placed in ice water bath, 10 ml of 2% solution of cold anthron was added on 2.5 ml of extract. In order to reach to room temperature mixture was heated to 95 º C for 7.5 min and read spectrophotometrically at 630 nm against glucose standards. Values were multiplied with 0.92 and the amount of starch was found [17].

Determination of proline

Bates, Waldren and Tear [19] ninhidrin method was applied for Proline extraction and determination.

1 -Approximately 0.5 g of fresh plant material were homogenized in 10 ml of 3% Sulfosalicylic acid. The homogenate was filtered Whatman No 2 filter paper

2 - 2 ml of filtrate was taken and poured in a test tube, then it was reacted with 2 ml glacial acetic acid and 2 ml acid-ninhidrin and was kept 100 ° C for 1 hour. The ice bath was used to terminate reaction

3 - reaction mixture was extracted with 4 ml of toluen and stirred 15-20 seconds

4 - The chromophore containing toluene was separated water phase and brought to room temperature and absorbance values were read at 520 nm

5 - Proline concentration was calculated according to the curve in which the standards was prepared by using of L-proline. The amount of proline in fresh plant sample was calculated created according to the Formula; [(µg proline / ml x ml toluene) / 115.5 mg / mmol] / [(g sample / 5)] = µmol proline / g fresh plant material. Experiments were performed with three replicates.

Determination of the amount of dry weight

The collected plant samples were weighed and hold in oven at 100 ° C for a week, and they were weighed a regular basis. The fixed value of weighing results was taken as the last weight and % dry weight content was calculated.

Observatıons and results

Observations

Gall samples were collected in April from Karaçayır. It was observed that galls turned into brown and firm structure in April. Gall has not yet started development in June. Because individuals of *Diplolepis mayri* consisting of last year and brown color. The insects leave their eggs in the flower buds in this month. The most prominent difference is observed between with gall individuals and without gall individuals in July, the fruits of individuals belonging to with gall early riped than the fruits of individuals without gall. In August, early maturation and the color change are seen fruits of individuals with galls. Fragmentation event took place in individuals with galls was observed very clear way.

In September, as observed in August, the ripening of fruits individuals with gall and color modification completed, the fruits belong to the individuals without galls color conversion of individuals was still not completed. When observations of ripe fruit with gall was made especially in September, itwas seen that the leaves around of the fruit with gall was fallen earlier

than other parts of plant. Starting around injection region the fruit slowly broken, and in this way the formation of the fruit gall was observed from fruit galls gathered in July.

Statistical analysis

Statististical analysis were performed using SPSS 16.0 for Windows. Tucey's Multiple Range Test was employed to determine the statistical significance of differences among the means.

Results

Leaf and fruit samples used for our research have been taken including the periods; June, July and August months before and after the development of gall. These months are considered the most appropriate terms for vegetative growth period of *R. canina.* Individuals without gall are defined as the control group in our study. Individuals with gall and without gall in control group were examined interms of changes of pigment (Chl-a, Chl-b and carotenoid), carbohydrates (starch and sugar), proline and the quantity of dry matter. Our goal was to investigate with the formation of gall, which physiological changes happened in *R. canina*. Leaf samples were only used for pigment analysis. On the other hand, for the analysis of carbohydrates and proline, in addition to using the leaf samples fruit samples were used in this study. Fruit samples are quite an important role in our study. Because we work gall samples which are fruit gall variety. In addition, the fruit samples used in study collected under three groups.

The first group was the control group cosisted of fruits without gall, the second groupv was fruits without gall belong to individuals with gall and the third group was fruits with gall belong to individuals with gall. The reason for doing such a grouping; classified as individuals with gall at the same time inlude fruits without gall. We aimed to investigate which changes were made by the feeding of larva in fruits with or without gall on the same plant. Moreover, the larvae was not brought in when working with fruit gall.

Chl-a, chl-b and carotenoid amounts in leaves of rosa canina members with or without gall

When with or without gall indivudulas of control group were compared in terms of aquantity of Chl-a and according to months: Gall development of individuals were not started in June, that's why, quantity of Chl-a in leaves of individuals with gall lower than the control group. On the other hand, in July it started to continue the development and maturation in

August. So, it has been determined that quantity of Chl-a in leaves of individuals with gall higher than that the control group. Moreover, the same situation was seen for Chl-b (Table 1).

The amount of carotenoids in the control group and the individuals with gall are compared acorrding to months. It was determined that in June, the amount of carotenoids in the control group was higher level than individuals with gall (Table 2). In July, with the formation of gall, the amount of carotenoids in individuals with gall was higher level than the control group showed that. In August, the amount of carotenoid in the leaves of the individuals with gall was still higher level than the control group (Table 2).

As a result, during July and August with the beginning of the development of gall, Chl-a, Chl-b and carotenoid content in leaves of individuals with gall were higher level than the control group (Table 1-2). There was no significant difference the rates of total Chlorophyll / carotenoid in June and July, while in August it was also found that the rates of total Chlorophyll / carotenoid in individuals with gall were high (Table 2).

Table 1. Chl-a and Chl-b amounts (µg / g wet weight) and rate of Chl-a/Chl-b in individuals with or without gall of *R. canina*.

Months	Parts of Plant	Chl-a	Chl-b	Chla/Chl-b Rates
June	Individual with Gall	25,80 ± 0.20a	8,21a ± 0.07a	3.14
	Individual without Gall	22.47 ± 1.43b	6,78b ± 0.36b	3.32
July	Individual with Gall	26,52 ± 0.43c	8,58a ± 0.18c	3.09
	Individual without Gall	28,26± 0.31d	9,64b ± 0.13d	2.93
August	Individual with Gall	26.75 ± 0.54c	8,80a ± 0.30e	3.05
	Individual without Gall	29,17 ± 0.31e	10,17b± 0.26f	2.87

[a]Means with the same letters do not significantly differ at 0.05 level

Table 2. The amount of carotenoids (µg / g wet weight), the total amount of Chlorophyll (µg/g wet weight) and total Chlorophyll / carotenoid rates in individuals (control group) with or without gall of *R. canina*.

Months	Parts of Plant	Carotenoid	Total Amount of Chlorophyll	Total Chlorophyll / Carotenoid Rates
June	Individual with Gall	7,65 ±0,22a	17,01 ± 0.14a	4.45
	Individual without Gall	6,22 ± 0.04b	14,63 ± 0.90b	4.70
July	Individual with Gall	7.31 ± 0.04c	17,55 ± 0.31c	4.80
	Individual without Gall	7.77 ± 0.08a	18,95 ± 0.22d	4.88
August	Individual with Gall	7.25 ± 0.08c	17,78 ± 0.42e	4.90
	Individual without Gall	7.79 ± 0.14d	19,67 ± 0.29f	5.05

[a]Means with the same letters do not significantly differ at 0.05 level

Changes of carbohydrate in leaves and fruits of individuals with or without gall of r. canina.

When changes the amount of sugar in individuals with or without gall were compared, differenrt results were obtained;

In June, the amount of sugar in leaves of individual without gall was found higher than leaves of individual with gall. In July and August with the formation of gall, the amount of sugar in leaves of individuals with gall was found more higher than individual without gall (Table 3). Starch content in leaves of individuals with gall was higher than individual without gall in June, in July and August starch content of individual without gall was higher than leaves of individuals with gall (Table 3).

In July, with the formation of gall, the amount of sugar in the leaves of individuals with gall was higher than individuals without gall (Table 3). Fruit samples collected in July and August were divided into groups, which were individual without gall-fruit, individual with gall-fruit without gall and individual with gall-fruit with gall.

The highest sugar content of fruit samples collected in July was individual with gall-fruits without gall and individual with gall-fruit with gall respectively, whereas in August, the least sugar content was determined in individual with gall-fruit with gall (Table 3).

Unlike June, the amount of starch was found high level in the leaves of individuals with gall (Table 3).

In July, the amount of starch of individual with gall- fruit without gall was high in comparison to control, in August the amount of starch has been found as the lowest in individual with gall- fruit with gall (Table 3).

In August, decreasing sugar and starch content of fruits with gall showed that carbohydrates were consumed during the development of the larvae which formed gall. Therefore, this supports the nutritional hypothesis. When the total carbohydrate amount was compared in June, there was not any difference between individuals with gall and control group.

In July, total carbohydrate content of leaves in the control group was found higher level than the leaves of individuals with gall (Table 3).

In short, with the formation of fruit gall, the amount of total carbohydrate in leaves of individual with gall did not increase in July. On the contrary, this month, compared to June total carbohydrate values of control group and leaves of individual with gall were lower.

As the cause of these results; with the beginning of the development of fruit, the metabolic product flow was seen from the leaves to the fruits.

When total carbohydrate modification fruit samples of July was examined, the most high carbohydrate value was found in fruits without gall of individuals with gall (Table 3). the amount of total carbohydrate in fruits with gall of individuals with gall was found higher than fruits without gall of individuals with gall. The reason for this can be connected to carbohydrate consumption of larvae.

In this month, the lowest value in terms of formation of total carbohydrates in fruits belongs to the fruits of individuals without gall. (Table 3).

Among the leaf samples taken in August, the highest total carbohydrate value has been found in the leaves of individuals with gall (Table 3).

Table 3. Sugar, starch and total carbohydrate content changes (m/g dry weight) in individuals with and without gall of R. canina (Values are given as the mean of three repetition ± standard error).

Months	Parts of Plant	Sugar Content Changes	Starch Content Changes	Total Carbohydrate Content Changes
June	Individual without Gall -Leaf	65.52 ± 1.48a	40.28 ± 0.62a	105.80 ± 2.10
	Individual with Gall -Leaf	57.91 ± 0.63b	47.91 ± 0.93b	105.82 ± 1.56
July	Individual without Gall -Leaf	45.13 ± 1.53c	30.15 ± 0.32c	75.28 ± 1.85
	Individual with Gall -Leaf	49.33 ± 2.93d	22.41 ± 0.07d	71.74 ± 3.00
	Individual without Gall - Fruit	49.89 ± 1.59d	20.96 ± 0.04e	70.85 ± 1.63
	Individual with Gall – Fruit without Gall	64.41 ± 1,98e	26.09 ± 1.01f	90.50 ± 2.99
	Individual with Gall – Fruit with Gall	61.71 ± 2.42f	26.75 ± 0.94f	88.46 ± 3.36
August	Individual without Gall -Leaf	46,56 ± 1.07g	26.06 ± 0.98g	72.62 ± 2.05
	Individual with Gall -Leaf	57,11 ± 1.98b	19.88 ± 0.47h	76.99 ± 2.45
	Individual without Gall - Fruit	69,65 ± 0.77h	17.66 ± 0.79i	87.31 ± 1.56
	Individual with Gall – Fruit without Gall	60,13 ± 1.13i	17.42 ± 0.11i	77.55 ± 1.24
	Individual with Gall – Fruit with Gall	57,27 ± 1.31b	15.25 ± 0.43j	72.52 ± 1.74

[a]Means with the same letters do not significantly differ at 0.05 level

Changes of dry weight

Leaf dry weight content of the control group in June was higher than leaves of individuals with gall (Table 4). This difference is consistent increase in the amount of sugar (Table 3).

It was determined that the amount of dry weight in leaves of individuals with gall in July was higher than the control group. In Samples of fruit, the most high-value was determined in individuals without gall-fruit. Dry weight content of fruits with gall of individual with gall was higher than fruits without gall of the same individuals (Table 4). The highest dry weight content in the leaves of the individuals with gall was determined in August just as July (Table 4). The highest dry weight content among fruit samples of august were the fruit without gall of individual with gall (Table 4). The most low value was found in fruits of individuals without

gall (Table 4). In general, there are similarities between the changes in dry weight content and the change in the amount of sugar (Table 3, 4).

Table 4. During the months, dry weight changes of individuals with or without gall (%)

Months	Parts of Plant	Total Dry Weight Changes (%)
June	Individual without Gall -Leaf	42.73 ± 0,04a
	Individual with Gall -Leaf	39,64 ± 0,09b
July	Individual without Gall -Leaf	40,62 ± 0,04c
	Individual with Gall -Leaf	42,40 ± 0,02a
	Individual without Gall - Fruit	25,07 ± 0,03d
	Individual with Gall – Fruit without Gall	23,88 ± 0,03e
	Individual with Gall – Fruit with Gall	24,21 ± 0,01f
August	Individual without Gall -Leaf	48,79 ± 0,03g
	Individual with Gall -Leaf	52.75 ± 0,06h
	Individual without Gall - Fruit	40,64 ± 0,04c
	Individual with Gall – Fruit without Gall	43,51 ± 0,03i
	Individual with Gall – Fruit with Gall	41.66 ± 0,07j

[a]Means with the same letters do not significantly differ at 0.05 level

According to months the quantity changes of proline in collected leaves and fruits of individuals with and without gall of rosa canina

When individuals with and without gall of *Rosa canina* were compared, a correlation was found between proline accumulation and formation of gall. When the comparison was made according to months, Because the formation of gall was not begin completely in June, the amount of proline in leaves of individuals with gall was found low level than the amount proline in leaves of individuals without gall (Table 5).

In July, with the start of the formation of gall, the proline content in leaves of individuals with gall was higher than the amount of proline content in leaves of individuals without gall (Table 5). In this month, amount of proline in fruits without gall of individuals

without gall was lower than amount of proline in fruits without gall of individuals with gall. On the other hand, in this month amount of proline in fruits with gall of individuals with gall was higher than both amount of proline in fruits without gall of individuals with gall and amount of proline in fruits without gall of individuals without gall (Table 5). In August, whit continuing the formation process of galls, the amount of proline in leaves of individuals with gall was higher than the amount of proline in leaves of individuals without gall as parallel with July (Table 5).

However, in this month, it was found that the amount of proline in fruits without gall of individuals without gall was lower level than both fruits without gall of individuals with gall and fruits with gall of individuals with gall. Also, in this month parallel with in July, the amount of proline in fruits with gall of individuals with gall was higher than fruits without gall of individuals with gall (Table 5).

Table 5. During the June, July and August, amounts of proline (µmol / g fresh weight) in plant parts of individuals with or without gall of *R. canina* L (Values are given as the mean of three repetition ± standard error).

Months	Parts of Plant	Content of Proline
June	Individual without Gall -Leaf	13.28 ± 0.78a
	Individual with Gall -Leaf	11.27 ± 0.88b
July	Individual without Gall -Leaf	4.42 ± 0.10c
	Individual with Gall -Leaf	6.34 ± 0.09d
	Individual without Gall – Fruit without Gall	39.50 ± 0.40e
	Individual witht Gall – Fruit without Gall	37.23 ± 0.21f
	Individual with Gall – Fruit with Gall	49.14 ± 0.70g
August	Individual without Gall -Leaf	3.79 ± 0.36h
	Individual with Gall -Leaf	5.53 ± 0.04i
	Individual without Gall – Fruit without Gall	13.75 ± 0.37a
	Individual witht Gall – Fruit without Gall	17.72 ± 0.38j
	Individual with Gall – Fruit with Gall	33.37 ± 0.33k

DISCUSSION and CONCLUSION

Galls the most well-known types of tumor in plant species wich are formed by viruses, bacteria, fungi, nematodes, ticks or insects by suppression of host plant tissues or by preventing the development of tissue growth and differentiation. Many changes occurring in response to gall-makers were determined in host plant tissues. These changes are to cover pH, polarity, nuclear and changes in nucleolar hypertrophy, the increase of free amino acids and sugars, amylase, protease, and production of the other hydrolytic enzymes [20, 21]. Although Insect galls are present all parts of plants, more than 75% of these galls are located on leaves of plants [22, 23]. Indeed, lots of the research focuses on physiological modifications made by gall of leaves in plants.

In this study we investigated gall forming on fruits of *R.canina* L. and physiological changes that occur with the formation of gall. Of course, it can not be expected to show the same response of plants against various stress conditions. Indeed, the formation of gall in our study included the biotic stress conditions. In this study during July and August with the formation of gall, noticeable improvement for Chl-a, Chl-b and carotenoid rates were determined in leaves of *R.canina* (Table 1, 2). There is not a general consensus on effect of gall-makers on host photosynthesis. As a matter of fact, this effect has been stated as positive or negative direction in some studies [24-27]. For example, Fay and colleagues stated that (25) galls of cynipid wasp created by *Antistrophus silphii* on *Silphium integrifolium,* increased photosynthesis, stomatal conduction and xylem water potential. We obtained the pigment and carbohydrate changes in host plants with the formation of gall. It was seen that study results of Fay and colleagues are parallel to our study. So it can be considered that with the formation of gall, the rate of photosynthesis increased in host plant.

In a study carried out by Yang et al. [28], ratio of Chl-a/Chl-b was found as 2.8 in infected leaves of *Machilus thungbergii* Sieb & Zucc (Lauraceae). Kramer and Kozlowski [29] reported that a normal amount of Chl-a was 2.3-2.5 times higher than Chl-b. In our study these rates in leaves of individuals with and without gall vary between 2.8-3.3 (Table 2). This results is similar to rates of healthy plant. According to the results we found that in individuals with gall this rate was higher in June. This raises the idea that Chl-a was synthesized faster than Chl-b or Chl-b was destroyed more quickly than Chl-b. In July, with the formation of gall, the rate of Chl-a/Chl-b in individuals with gall was lower than the control group. This stiuation suggest that Chl-b was synthesized faster than Chl-b. Gall maturation continued in August compared to July revealed that a parallel situation in terms of the rate of Chl-a/Chl-b (Table 1).

Total Chlorophyll / carotenoid ratio in a healthy plant has been reported that 4.8 [30]. Total Chlorophyll / carotenoid ratios obtained in this study were parallel to the healthy plant. Although a significant difference between individuals with gall and control group was determined in terms of this ratio, with the formation of gall a significant difference was not observed (Table 2). However, it was seen that with the start development of gall in July, these rates were observed as very close to each other. This rate in the months of June and August, is higher in individuals with gall than the others (Table 2). Highness of this ratio in individuals with gall can be suggested that destruction of carotenoid in these individuals is more quickly.

How sugar affects the amount of biotic stress conditions in leaves was investigated by many scientist. In a study made by Sárdi et al. [31], after infection of bean leaves by *Pseudomonas savastanoi* pv. *Phaseoicola*, it was found the decrease in glucose concentration in leaves. Formation of gall is a biotic stress factor for plants. In our study, with the formation of gall in July, the amount of sugar was found higher in leaves of individuals with gall than the other groups (Table 3). On the other hand, because of the fact there was not any gall development in June, the amount of sugar in the leaves of the control group was higher than the other groups (Table 3). When fruit samples were examined, in July it was seen that the fruits without gall of individuals with gall had higher amount of sugar than the fruits of control group. These results seem to support the idea that fruits without gall of individuals with gall matured earlier than the control group. Fruit ripening includes enzymatic degradation of cell walls, hydrolysis of starch, sugar accumulation, softening originated from organic compounds loss including organic acids and tannins, and a loss of Chlorophyll with the color modification [32]. Fruits of individuals with gall were ripened more quickly. So, it can be suggested that ethylene synthesis, which accelerates the ripening of fruits, was raised in these individuals. In one study of Scareli-Santos [33], chemical and morphological changes on host plants of two different gall constructor were investigated and it has been found out that these species have created different impacts on hosts.

The diet hypothesis especially proposed by Price, et al. [34], was tested in this study and not found any relationship between the formation of gall and this nutrition hypothesis. On the other hand, in July and August especially accelerated gall maturation decrease the amount of sugar in fruits with gall. Hence, it can be suggested that this decrease support the nutrition hypothesis. Moreover, with the increase in nutrient requirements of larval development may be the reason for this decline (Table 3).

In a study of Yang et al. [28], in which they studied the comparison of tilakoid morphology, pigment-protein complexes, Chlorophyll biosynthesis and degradation pathways in maturated galls and infected leaves of *Machilus thunbergii* Sieb & Zucc (Lauraceae) host plant. They have found that due to some components were lost with the formation of gall suggested, there was not much, pigment-protein complexes during the formation of gall. Also, this researchers in the same study determined that degradation of Chlorophyll in galls and infected leaves was happened with different ways.

Although there are lots of researces and the identifying sources on the insect galls living on plants [35], the knowledge on the formation physiology and development physiology of galls is insufficient. However, the formation of gall is presumed to be caused by a insect origin chemical compound [36]. Most investigator found that organs of such insects or extracts of gall constructive insects caused abnormal growth in plant tissues [37, 38]. Different studies of Leatherdale [38], prepared extracts of such gall-makers. This researcher injected extract of *Dasyneura urticae* with help of a very fine injector in immature leaves of *Urtica dioica.* All insect extraction experiment, only 12 of 150 injection, the head of larval extraction experiment, 9 out of 50 injection led to abnormal growth. Water applied only in the control group was not observed in abnormal growth.

In reality, of course, such injections were unfulfilled, but the production of a typical gall is not happened as uncertain. The formation of gall is likely to be done that leaving the right place and right amount of chemicals [35]. As amatter of fact, our observations, especially in certain regions on the fruits on which brown spots formed. We think that those regions were injection sites in which *Diplolepis mayri* left it's eggs with the help of ovipositor. In our observations we also observed that fruit crumbling was around these regions.

A study made by Vuorisalo et al. [39], stated that as a result of metabolic product competition between the developing gall and leaf, leaf loss was happened and this loss increased indirectly the performance of gall. In our observations especially made in September, loss of leaf was found from fruits with gall of the shoots these seem to support this work. Accumulation of L-proline (Proline) in different environmental stress conditions has been shown in many studies [40]. Proline is a hydrophilic amino acid and it is thought that high hydrophilic values cause the better enzyme-substrate reaction.

There are few studies about proline accumulation in plants for response to biotic stress conditions. In the other study made with leaf tissue cultures and galls of *Phylloxera* formed on grape leaves was found that extracts of gall contained more proline than extracts of leaves [41].

In particular there are few studies showing the relationship between proline accumulation and the formation of gall. So our study demonstrates the importance for this area. The results we have obtained showed that there was a significant relationship between proline accumulation and the formation of gall in leaves and fruits. Especially this accumulation was very high level in fruits with gall of individuals with gall. So, it can be suggested that stress center at the host plant is in this region (Table 5). Although, most of the studies focuse on anatomy and morphology of insects and gall made by these insects, Wales [22], there are few studies focuse on physiological and biochemical changes of plant.

Finally in this study, significant differences have been determined for the changes in the amount of pigment, carbohydrates and proline of members with or without gall of *Rosa canina* L. Our findings can be helpful in future studies addressing other effects of plant galls.

REFERENCES

[1] Cornell, H.V., 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera):why and how? American Midland Naturalist 110, 223-224.

[2] Ronquist, F. and Liljeblad, J., 2001. Evolution of the gall wasp-host plant association. Evolition, 55, 2503-2522.

[3] Bronner, R., 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiidae. In Biology of Insect Galls (eds J.D. Shorthouse & O. Rohfritsch), pp. 118-140, Oxford University Press, New York.

[4] Bayer, M.H., 1992. Biochemical modification of the phenotype in cynipid galls. In Plant Galls: Organism, Interactions, Populations (ed. M.A. J. Williams), pp. 429-446. Clarendon Pres. Oxford.

[5] Bagatto, G., Paquette, C. and Shorthouse, J.D., 1996. Influence of galls of Phanacis taraxaci on carbon partitioning within common dandelion, Taraxacum officinale. Entomologia Experimentalis et Applicata, 79, 111-117.

[6] Berland, L. and Bernard, F., 1951. Ordre de Hymenoptères. In: Traitè de Zoologie, no.10 (ed. P. Grassè), pp. 771-1276, Mason, Paris.

[7] Mani, M. S., 1964. Ecology of plant galls. Dr. W. Junk, Publishers, The Hague, 434p.

[8] Bequaert, J., 1924. Galls that secrete honeydew: a contribution tothe problem as to whether galls are altruistic adaptations. Bulletin of the Brooklyn Entomological Society, 19, 101–124.

[9] McCrea, K.D., Abrahamson W.G. and Weis A.E., 1985. Goldenrod ball gall effects on Solidago altissima: 14 C translocation and growth. Ecology, 66, 1902-1907.

[10] Weis, A.E., Walton, R. and Crego, C.L., 1988. Reactive tissue sites and population biology of gall makers. Annual Review of Entomology, 33, 467-486.

[11] Hartley, S.E. and Lawton, J.H., 1992. Host plant manipulation by gall insects: a test of the nutritionhypothesis. Journal of Animal Ecology, 61, 113-119.

[12] Abrahamson, W.G. and Weis, A.E. 1987. Nutritional ecology of arthropod gallmakers. Nutritional Ecology of Insects, Mites, and Spiders (ed. by J. G. Rodriquez and F. Slansky, Jr), pp. 235–258, Wiley, New York.

[13] Price, P.W., Fernandes, G.W. and Waring, G.I., 1987. The adaptive nature of insect galls. Environmental Entomology, 16, 15-24.

[14] Nyman, T. and Julkunen-Titto, R., 2000. Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. Proceedings of the National Academy of Science, 97, 13184-131817.

[15] Özbek, H., Güçlü, S. and Tozlu, G., 1999. Biology and natural enemies of Diplolepis mayri Schld. (Hymenoptera: Cynipîdae), a pest of *Rosa canina* L. in Erzurum Province . Türk. Entomol. Derg., 23(1) , 39:50

[16] Lichtenthaler, H.K. and Wellburn, A.R., 1983. Determinations of total carotenoids and chylorophylls a and b of leaf extracts in different solvents. Botanisches institut der Universitat, Kaiserstran βe 12, Postfach 6380.

[17] McCready, M.R., Guggolz, J., Silviera V. and Owens S.H., 1950. Determination of starch and amylose in vegetables. Anal. Chemistry., Vol. 22, pp. 1156- 1158.

[18] Ebell, L.F., 1970. Variation in total soluble sugars of conifer tissues with method of analysis. Phytochemistry, Vol. 8, pp, 227-233.

[19] Bates, L.S., Waldren R.P. and Teare I.D., 1973, Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205-207.

[20] Mani, M.S., 1992. Introduction to Cecidology. In J. D. Shorthouse and O. Rohfritsch (eds.), Biology of insect-induced galls. Oxford University Press, Oxford, England, pp. 1-7.

[21] Rohfritsch, O., 1992. Patterns in gall development. In J. D. Shorthouse and O. Rohfritsch (eds.), Biology of insect-Induced Galls. Oxford University Press, Oxford, England, pp. 60-86.

[22] Dreger-Jauffret F. and Shorthouse, J.D., 1992. Diversity of gall-inducing insects and their galls. In J.D. Shorthouse and o. Rohfritsch (eds.), Biology of insects- Induced Galls. Oxford University Pres, Oxford, England, pp. 8-33.

[23] Yang, M. M. and Tung, G.S., 1998. The diversity of insect-induced galls on vascular plants in Taiwan: a preliminary report. In G. Csóka, W. J. Mattson, G.N. Stone, and P. W. Price (eds.), The Biology of Gall-Inducing Arthropods. Gen. Tech. Rep. NC-199. St. Paul, MN: USDA, Forest Service, North Central Forest Exoeriment Station, pp. 44-53.

[24] Andersen, P.C. and Mizell, R.F., 1987. Physiological efects of galls induced by Phylloxera notablilis (Homoptera: Phylloxeridae) on pecan foliage. Environ. Entomol. 16, 264-268.

[25] Fay, P.A., Hartnett, D.C. and Knapp, A.K., 1993. Increased photosynthesis and water potentials in Silphium integrifolium galled by cynipid wasps. Oecologin 93, 114-120.

[26] Bagatto, G., Paquette, C. and Shorthouse, J.D., 1996. Influence of galls of Phanacis taraxaci on carbon partitioning within common dandelion, Taraxacum officinale. Entomologia Experimentalis et Applicata, 79, 111-117.

[27] Larson, K.C., 1998. The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. Oecologia. 115, 161-166

[28] Yang, C., Yang, M., Hsu, J. and Jane, W., 2003. Herbivorous insect causes deficiency of pigment-protein complexes in an oval-pointed cecidomyiid gall of Machilus thunbergii leaf. Bot. Bull. Acad. Sin., 44, 315-321.

[29] Kramer, P.J. and Kozlowski, T.T., 1979. Physiology of Woody Plants. pp. 167.

[30] Goss, A.J., 1972. Physiology of Plants and Their Cells. pp. 137.

[31] Sárdi, E., Velich, I., Hevesi, M. and Klement, Z., 1999. Ontogenesis- and Biotic Stres-Dependent Variability of Carbonhydrate Content in Snap Bean (Phaseolus vulgaris L.). Z. Naturforsch., 54c, 782-787.

[32] Bidwell, K., 1979. Physiologia Plantarum. Vol:46. pp. 299-306.

[33] Scareli-Santos, C., 2002. Avaliação de sistema galhador-planta hospedeira em ambiente de cerrado: aspectos morfo-anatômicos e fitoquimicos. Acta. Bot. Bras., 16(4), 501-503.

[34] Price, P.W., Fernandes, G.W. and Waring, G.I., 1987. The adaptive nature of insect galls. Environmental Entomology, 16, 15-24.

[35] Felt, E. P., 1940. Plant Galls & Gall Makers. Comstock Publishing Co., Ithaca, N. Y. pp. 3-32.

[36] Plumb, G.H., 1953. Formation and development of the Norway Spruce gall caused by Adelges agabeyetis L. Conn. Agric. Exp. Sta. Bull. 566, New Haven, Conn.

[37] Anders, F., 1958. Aminosäuren als gallenerregende Stoff der Reblaus (Viteus (Phylloxera) vitifolii Shimer). Experentia, 14, 62-63.

[38] Leatherdale, D., 1955. Plant hyperplasia induced with a cell-free insect extract. Nature 175, 553-554.

[39] Vuorisalo, T., Walls, M. and Kuitunen H., 1990. Gall mite (*Eriophyes laevis*) infestation and leaf removal affect growth of leaf area in black alder (*Alnus glutinosa*) short shoots. Oecologia, 84, 122-125.

[40] Delauney, A.J., Hu, C.A., Kishor, P.B. and Verma, D.P., 1993. Cloning of ornithine delta aminotransferase cDNA from Vigna aconitifolia by transcomplementation in Escherichia coli and regulation of proline biosynthesis. J. Biol. Chem., 268(25), 18673-8, PMID. 8103048.

[41] Warick, R.P. and Hildebrandt, A.C., 1966. Free Amino Acid Contents of Stem and Phylloxera Gall Tissue Cultures of Grape. Plant Physiol., 41, 573-578.