INCREASE IN GALL ABUNDANCE AND DIVERSITY IN *POPULUS FREMONTII* STANDS

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ABSTRACT

The importance of galling insect communities and resultant effects of galling on *Populus* sp. is well documented in previous studies, but there has been a paucity of studies on galling insect communities on *Populus fremontii*. This study hypothesizes that: as stand age increases, the abundance and variety of galls increases; more individual galls per unit of leaf area can be found as stand age increases; and that gall diversity will also increase as a function of leaf area. Also, we define branch galls on *P. fremontii* trophic structures. Trees were sampled in the experimental *P. fremontii* stands at the Cibola Nation Wildlife Refuge in southwest Arizona. Our results show a strong positive relationship between tree age of *P. fremontii* and gall abundance and diversity. We found branches of six year old *P. fremontii* with branch galls tend to have greater diameters than ungalled branches on the same trees. This study points to need for further research on galling insects' relationship with indeterminate growth characteristic to *P. fremontii*.

INTRODUCTION

The importance of galling insect communities and resultant effects of galling on *Populus* sp. is well documented in previous studies concerning *Populus angustifolia*

(Whitham 1979, Moran and Whitham 1988, Kearsley and Whitham 1989, Moran and Whitham 1990, Whitham et al. 1996, Kearsley and Whitham 1997) and *Populus* hybrids (Floate et al. 1997, Waltz and Whitham 1997), but there has been a paucity of studies on galling insect communities on *Populus fremontii*. The focus of this study is to quantify gall abundance and diversity in *P. fremontii* along an age gradient as well as clarify the relationship between stand structure and gall abundance and diversity. This study was initiated with the hypothesis that as stand age increases, the abundance and variety of galls increases. Additionally, this study proposes that more individual galls per unit of leaf area can be found as stand age increases. Gall diversity will also increase as a function of leaf area.

Study Organisms

Pemphigus aphids have a complex life cycle consisting of many generations (Harper 1959, Harper 1963, Moran and Whitham 1988). *Pemphigus betae* aphids overwinter as eggs in bark crevices of *P. angustifolia* (Moran and Whitham 1988), while *P. fremontii* supports overwintering *Pemphigus populicaulis* and *Pemphigus populitransversus*. These eggs hatch in the spring concurrent with early *Populus* leaf production. Each fundatrix (wingless female stem-mother) emerges and makes her way towards the emerging leaves. *P. populicaulis* bite the petiole at the leaf junction, provoking gall formation at that site. *P. populitransversus* trigger gall formation at the mid-point of the petiole. Each fundatrix enters the gall and reproduces parthenogenetically, producing up to 300 fundatrigenia (Whitham 1979).

Two major types of galls are initiated by two categories of insects. Open galls are the result of insects with piercing, sucking mouthparts interacting with susceptible plants. This sort of insect includes aphids, mites, psyllids, and scales. These insects reproduce inside the galls, culminating in a new generation which exits the gall leaving the remnant gall structure with a craterous opening. Closed galls are associated with insects with chewing mouthparts such as larvae of beetles, flies, moths, and wasps utilizing galls as shelter and food. These insects do not reproduce inside of galls, and leave behind small round holes after they exit. Alternate structural doorways are less common but can result from insectivorous parasitoids and hyperparasites. Petiole galls are seasonal, while stem galls persist as structures that may be shelter or ovipository sites by other insects. In the tradition of recognizing keystone species, this study proposes branch galls be recognized as keystone structures.

METHODS

Trees were sampled in the experimental *P. fremontii* stands at the Cibola Nation Wildlife Refuge in southwest Arizona between April 20th and 25th, 2006 (Figure 1). Comparison was made between three different age stands of *P. fremontii* with even ages of one, three and six years old. These stands were codified as stands 1, 2 and 3. Stand 1 and 2 were each approximately 20 acres. Stand 3 was of smaller acreage and not uniformly rectangular as were the other two (Figure 2). The corner points and the center of each plot were recorded using a Garmin ETREX hand held GPS unit with an accuracy of ≥ 14ft.

Within each stand, four plots were established. Using the center of each stand as a starting point, the locations of the four plots were designated by a random number chart. Once each plot center was designated, a measurement was taken 10 meters out in each cardinal direction (North, South, East, and West) to create a circular plot with a diameter of 20 meters, giving each plot an area of 314.16 m^2. The four "cardinal trees" plus the center tree of each plot were sampled for gall presence and type. Random branches with various diameters were surveyed until enough branches had been sampled to reach a total of 5 cm of branch diameter. The number of branch, petiole, and leaf galls was recorded. The data collected were used to determine the relationship between stand age and gall abundance/presence.

Leaf area was calculated for each branch sampled using equation; Leaf Area (LA) = 1990.49*(branch diameter)^2 + 3115.30*(branch diameter) with $r^2 = 0.81$, $p < 0.0001$ (Fischer et al.2004). The average number of galls per branch was divided by the average leaf area per branch to determine the number of galls per cm^2.

Data were quantified and multiple regressions were performed using Microsoft Excel 2003 and SPSS 13.0. Exponential curves best describes many of the data sets. However, some data points contained zeros, making exponential regressions impossible. To correct for this, 1 gall was added uniformly to every data point for number of galls per 5 cm of branch diameter and number of branch galls per 5 cm of branch diameter. Similarly, the line function that best describes the number of galls per unit of leaf area is also an exponential curve. To correct for this, 0.00001 galls were added uniformly to every data point for number of galls per cm^2 of leaf area.

RESULTS

The data show significant differences between gall abundance and diversity in stands of different ages. In the samples taken from the stand 1, no galls of any type were observed. Branch galls were first observed in stand 2. Stand 3 showed a marked increase both in the number and type of galls when compared with the two younger stands (Fig. 3.). Our findings elucidated a relationship between tree age and the number of galls per 5 cm of branch diameter (Fig. 4). An SPSS exponential regression calculated a p value of <0.001, affording the rejection of a null hypothesis of no relationship between stand age and the number of galls. We found a similar increase in the number of branch galls and stem galls across an age gradient, also with a p value of <0.001 (Fig. 5). Unlike petiole galls, branch galls continue to accumulate throughout the trees life and will persist while the branch remains intact. For this reason, projections can be made for future branch gall abundance. Calculations were also made comparing the average number of galls per cm^2 of leaf area per tree (Fig. 6.) which also increased with stand age. In addition to exploring the original hypotheses, this study also found that the branch gall abundance increased as a function of branch diameter (Fig. 7.).

DISCUSSION

Our study shows a strong positive relationship between tree age of *P. fremontii* and gall abundance and diversity. Older *P. fremontii* individuals host not only more galls overall, but more types of galls. Gall accumulation will continue as trees age, pointing to the possibility of an increase in the number of galls per tree throughout the life of the tree.

Petiole galls formed by *Pemphigus populicaulis* and *Pemphigus populitransversus* were found in the oldest stand in the experiment which had external conditions in common with the younger two stands save stand age. It is certain that *Pemphigus* aphids discriminate in choosing host plants for overwintering and spring petiole gall formation (Rhomberg 1984, Kearsley and Whitham 1997, Moran and Whitham 1990), but exactly what quality or qualities that come with tree maturity they prefer is unknown. Defensive chemistry may have an influence on *Pemphigus* aphid preference of older *Populus*; chemical analysis is necessary to test this hypothesis. Phenolics, carbon-based compounds that include flavinoids, phenolic glycosides, and tannins, are found *Populus* species. Phenolic glycosides, a group including salicin, salicortin, tremuloiden, and tremulacin, have been linked in a negative relationship to *P. betae* in *Populus angustifolia* (Zucker 1982). Our findings would support a similar negative relationship between phenolic glycosides, *P. populitransversus* and *P. populicaulis* as expressed through petiole gall location choice on *Populus fremontii*.

A study on a branch galling midge found higher gall presence with increased branch diameter (Prado and Vieira 1999). This Brazilian study also observed greater foliar mass found on branches with galls of the same age in the same habitat than without galls. Galled branches were larger than ungalled branches of the same age. Our study corroborates these findings with data showing that branches of six year old *P. fremontii* with branch galls tend to have greater diameters than ungalled branches on the same trees (Figure X). There are two possible hypotheses for these relationships: the ovipositing females are choosing more vigorous, fast-growing branches, and they remain more vigorous even after being galled (Price 1991); or that galling increases branch growth rates via resource regulation (Craig et al. 1986). A 1997 study reveals the *P. betae* preferentially colonizes branches of a host tree featuring more mature phenotypes, lending weight to the plant vigor hypothesis (Kearsley and Whitham 1997). This galling-insect selectivity may prove relevant in its possible consequences on *Populus sp.* indeterminate growth patterns.

Of the three different types of branch galls observed in the oldest *P. fremontii* stand, two are found, though sparingly, on three year-old *P. fremontii* individuals. The third branch gall type is of unknown origin and was first observed at the study site in the course of our research (verified by L. Hagenauer, Northern Arizona University, and R. Bangert, Northern Arizona University). The two familiar branch galls are host not only to the originating parasites, but parasitoids and, for the closed branch gall, hyperparasites, namely egg-laying wasps (Hagenauer, personal communication).

Insect diversity increases drastically in proportion to increases in gall type as each gall may provide food, shelter, a safe repository for eggs, or any combination of these resources (Ohgushi 2005).

The experimental gardens used in this research are situated in the floodplains of the Colorado River, which is a rich hydrarch. However, the floodplains are part of the Sonora Desert, and the average rainfall is less than two inches per year. This obviates nutrient leaching from the soil, maximizing the efficacy of each biological input, such as avian feces and carcasses, thus increasing nutrient cycling through the parasitized trees. While galling insects are parasites, that label may mislead one to believe that there are no possible benefits to the host. On the contrary, an increase in galling insects leads to an increase in galls, leading to an increase in overall insect abundance and diversity and the related increase in avian abundance (Spofford 1977, Haigler et al. 2006). Galls as keystone structures evidence a trophic cascade of valuable inputs in the desert ecosystem of which *P. fremontii* is a part.

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Fig. 1. Location of study site.

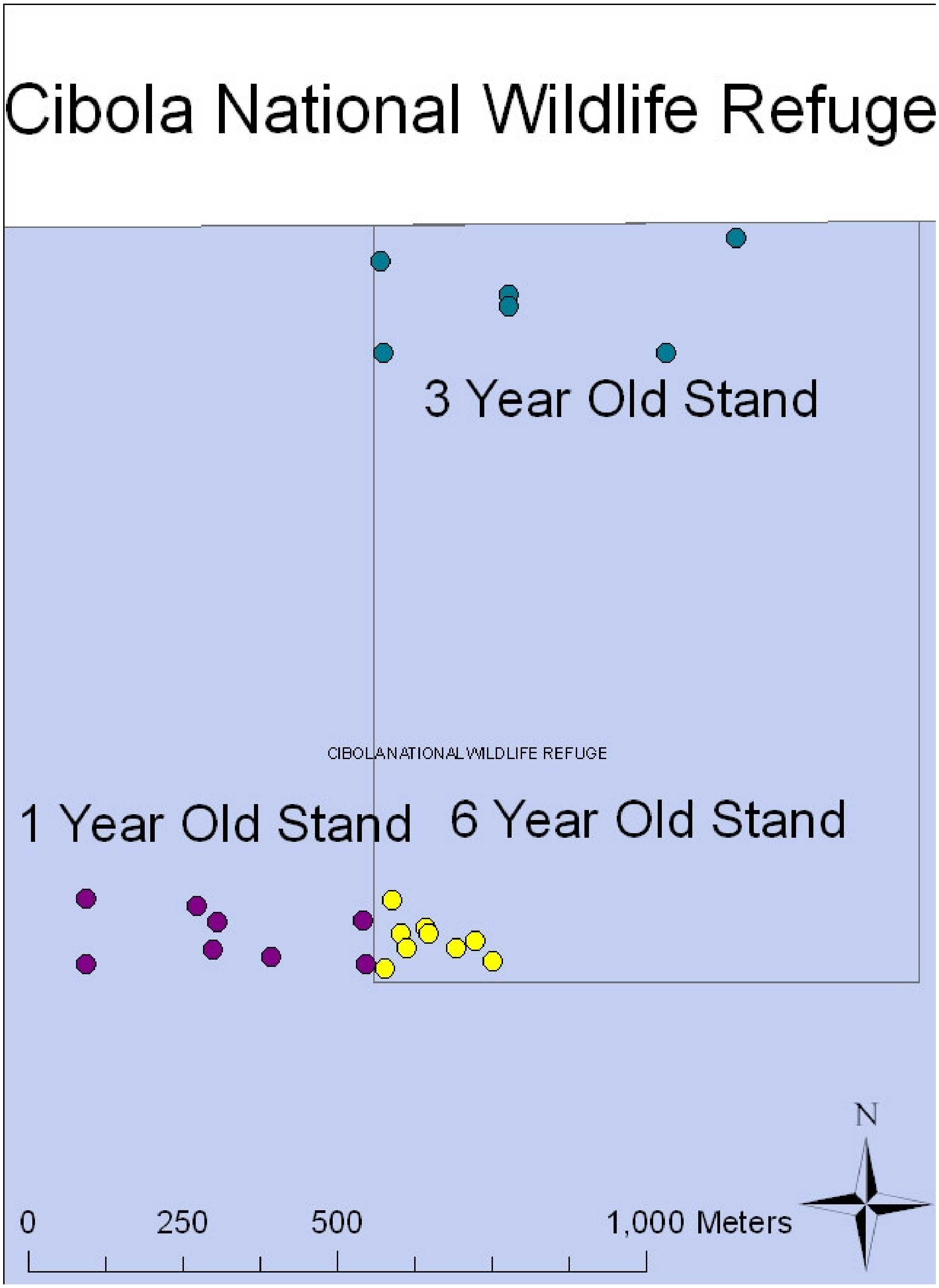

Fig. 2. Map of study site.

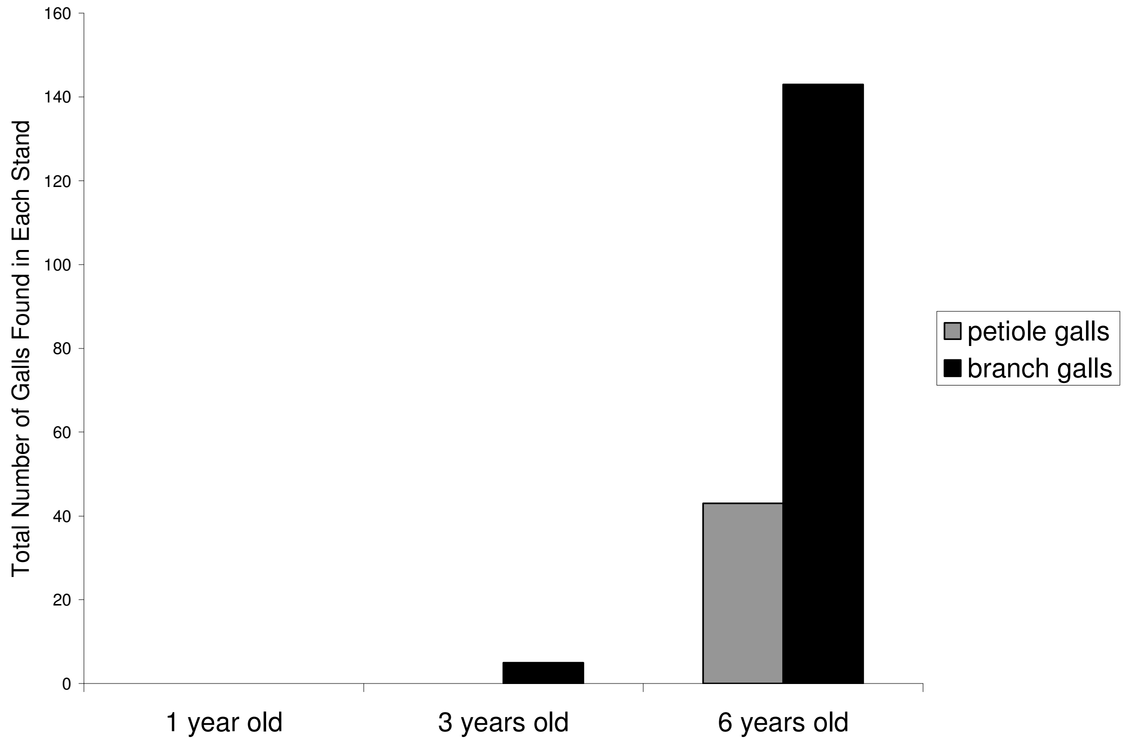

Fig. 3. Bar graph of total petiole galls and branch galls found on samples in each experimental stand of *P. fremontii*. n=60.

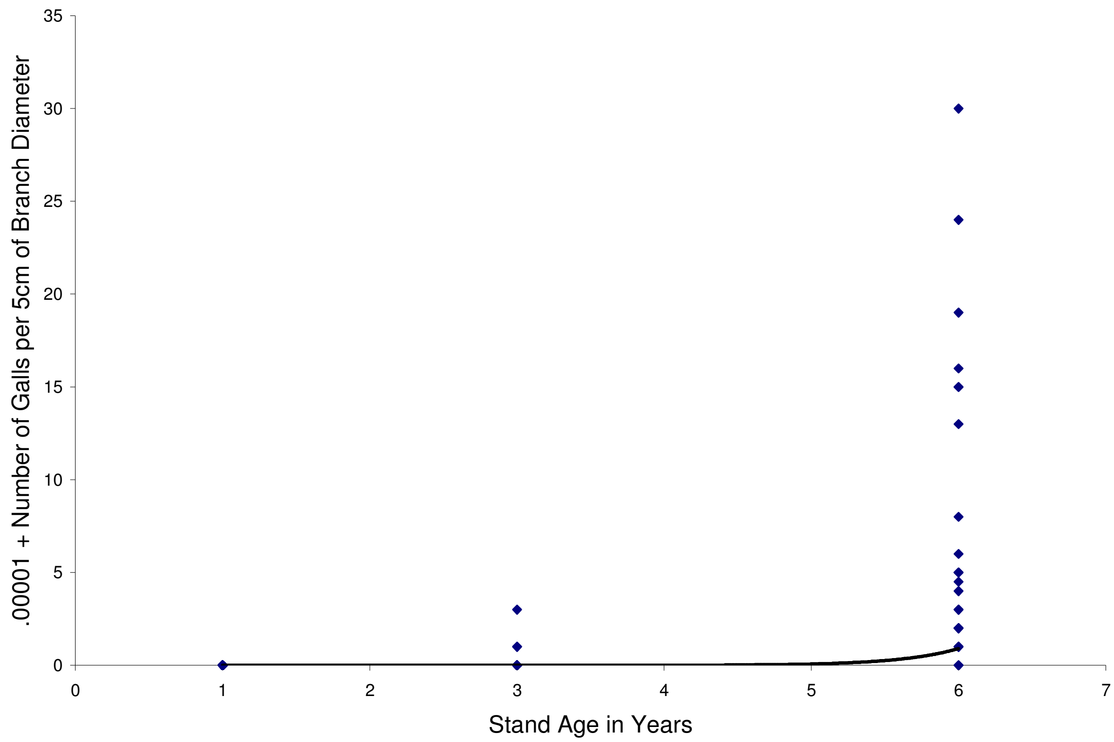

Fig. 4. Regression analysis showing relationship between tree age and number of galls + 0.00001 found per 5 cm of branch diameter ($y= 2E\text{-}07e^{2.5276x}$, $R^2 = 0.6889$, P value = <0.001).

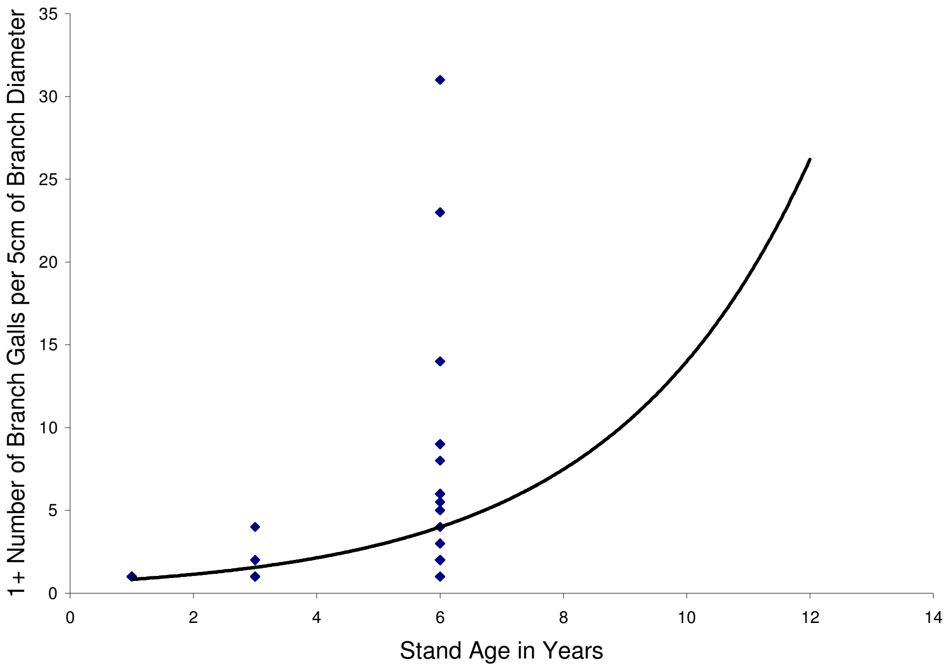

Fig. 5. Regression analysis indicating the relationship between *P. fremontii* stand age and number of branch galls found per 5 cm of branch diameter (y = 0.6085e0.3135x, R^2 = 0.5172, P value = <0.001). The regression analysis past 6 years is an estimated projection for older stands.

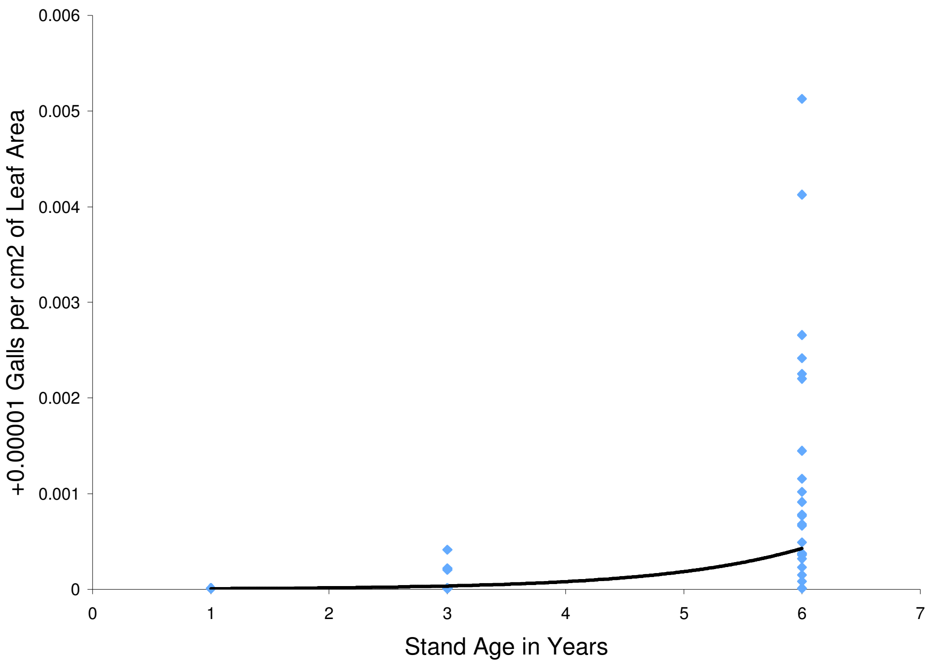

Fig. 6. Regression showing the relationship between galls per cm^2 of leaf area and tree age (y = 3E-06e0.8403x, R^2 = 0.6618, P value = <0.0001).

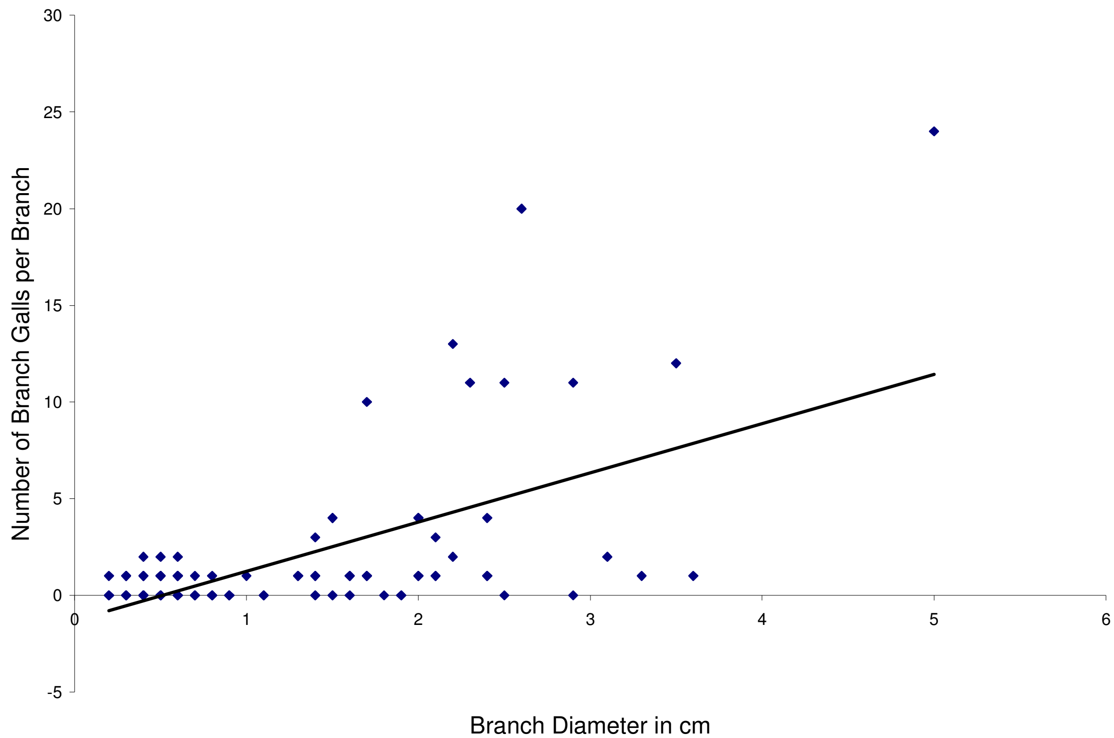

Fig. 7. Linear regression analysis showing positive relationship between branch gall abundance and larger branch diameter.(y=2.5455x-1.296, R^2=0.3755, P value = <0.0001)