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ENHANCING MULBERRY (*Morus alba* Linn.) CANOPY REGROWTH USING GIBBERELLIC ACID (GA3) AND HEIGHT PRUNING TECHNIQUES

Armando D. Diga[1]

[1]College of Agriculture Forestry Engineering and Development Communication (CAFED)
Ilocos Sur Polytechnic State College) Santa Maria, Ilocos Sur, Philippines
dollente_armando46@yahoo.com

Abstract

Effects of pruning and growth regulator application on plant regrowth are significant to mulberry plant production. The utilization of different levels of gibberellic acid concentration(GA3) and height pruning technique investigated in mulberry 'Batac' variety in a mulberry plantation located in Ilocos Sur to determine its effects on plant regrowth in terms of the number of days to budding, number, and length of shoots, leaf senescence, biomass herbage, leaf growth ratio, and net assimilation rate, There were two height pruning strategies implemented like 0.50m and 0.75m and four different GA3 concentration like 0 ppm, 100 ppm, 200 ppm, and 300 ppm laid out in factorial randomized complete block design with three replications. The investigation revealed that mulberry plants applied with 300 ppm gibberellic acid yielded effective extensive leaf area in contrast to other treatments, including the treatments with no GA3 application. The application of GA3 on the mulberry plant after pruning either 0.50m or 0.75m advances plant growth in leaf augmentation and leaf deterioration. 300 ppm GA3 concentration produced the highest biomass herbage and yielded the highest mulberry foliage but enhanced leaf senescence tantamount to its speedy decline. There is no significant interaction between pruning heights and the different concentrations of GA3 applied to the mulberry plants. The highest profit was established by the highest concentration of GA3 with the highest net farm income, benefit-cost ratio, and rate of return per ¼ hectare mulberry farm.

Keywords: *height pruning, regrowth, assimilation rate, leaf deterioration, leaf growth ratio. Mulberry*

I. INTRODUCTION

The global silk production largely depends on superior silkworm hybrids, improved rearing technology, nutritious mulberry leaf (feed to silkworm), a disease-free environment for rearing, and improved cocoon quality reeling system for higher quality raw silk production (Chauhan &Tayal, 2017). Silk production from mulberry mainly depends upon leaf yield. Silk products come from the silk filament produced by the highly domesticated silkworm (Bombyx mori Linn.), feeding solely on leaves of mulberry plants (Morus alba). Thus, mulberry cultivation is an integral part of and plays a significant role in the sericulture industry. The volume of available mulberry leaf determines the scale of silkworm production. The quality and quantity of mulberry leaves also affect the growth and development of silkworms and the quality and amount of cocoons produced.

Plant regulators are organic compounds synthesized in specific plant parts in small quantities and transported to the place of requirement leading to change in physiological responses (Geetha & Murugan, 2017). gibberellic acid is a phytohormone that stimulates cell enlargement and cell division and stimulates rapid stem and root growth, inducing mitotic division in some plants' leaves. Several scientific works revealed that the application of GA3 at 0.05 to 1.0 mg/l along with BAP stimulates shoot elongation and senescence under limited moisture conditions but depresses leaf enlargement (Ohnishi, 1986, Suzuki et al., 1989, Kumar et al., 1990). The height of plants increased, and the number of days increased with increasing concentration of GA_3. GA_3 at 100, 200, and 400ppm concentrations decreased female production and increased male and mixed flowers, respectively (Koyuncu, 2005). A similar study revealed that applying phosphorus with 0-200 ml/l of gibberellic acid concentration improve leaf area, chlorophyll content, and carbohydrates of grapes (Noori et al., 2018).

Pruning or training in mulberry cultivation is one method of increasing yield and quality. Among the forms of training used is based on height from the soil surface (lowcut (15-30cm), medium-low cut (30-50cm) and medium-cut (50-100cm), fist shape training, and lateral branch training (Machii et al., 2000). Studies have shown that pruning stimulates stem growth and increases the number of twigs and leaves of mulberry (Xue, Su & Chen, 2015). Growth and fruit quality improved using increasing pruning severity (Pawan et al., 2017). Yearly pruning facilitates the harvesting, cultural and plant protection operations. Pruning is essential to get a continuous economic yield of good quality leaves and fruits. Pruning methods on mulberry determines canopy development and leaf regrowth that influence the quality of leaves available for silkworm feeding.

To date, studies showing the effects of the application and concentration of GA3 coupled with the pruning technique (height of pruning) are limited. Hence, this study aimed to investigate the effectiveness of the different levels of concentration of gibberellic acid and mulberry pruning of various heights on canopy regrowth and biomass yield of mulberry for cocoon production.

Corresponding author:dollente_armando46@yahoo.com
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II. MATERIALS AND METHODS

Experimental site and plant material

The experiment was carried out in the mulberry farm site at a farmer's field in Limmansangan, Margaay, Narvacan, Ilocos Sur, with 450 square meters. Mulberry plant 'Batac' variety was used as planting material.

Experimental condition

For this experiment, ten years old mulberry bush plantation was selected. According to the silkworm rearing season, the mulberry garden was pruned four times in a year, each after six months. For this experiment, the gibberellic acid application and pruning were made from September to November. 2020.

Experimental Design

The experiment was laid out in a Factorial Randomized Complete Block Design assigning pruning height to Factor A and level of gibberellic acid concentration to Factor B with three replications. First, the farm lot (planted with the 'Batac' variety) is divided into three equal blocks. Then, each block was further subdivided into eight (8) identical plots to accommodate the eight (8) treatment combinations assigned at random across the fields within the block.

Treatments

There are two-factor treatments for this experiment as follow:

P- Pruning height was initiated in the experimental plants at 0.75m (P1) and 0.50m (P2).

G- Gibberellic acid concentrations were applied at a rate of 0 ppm (G0), 100 ppm (G1), 200 ppm (G2), and 300 ppm (G3) used only as a foliar spray.

Experimental Procedure

The treatments were randomly assigned in each plot. First, pruning of plants at a specific height was done in September before applying Gibberellic acid (GA3). Then, the different gibberellic acid concentrations were sprayed twice. The first spraying operation was done immediately after pruning, and the second application was done the third week after pruning when leaf flushes emerged. In all the treatments, 30 ml/100 L-1 of a non-ionic surfactant (Extravon®, Syngenta Agro S/A) was used to improve wetting and spray distribution.

Data gathered

According to the treatments, the data was collected one and six weeks after the last GA3 application. As a result, the data collected as follow:

The number of days to budding or leaf flushing. The number of days from pruning to 75 percent budding or leaf flushing was monitored and recorded from all the plots.

The number of leaf flushes or buds per plant. The number of leaf flushes or buds per plant was counted and recorded five (5) days after leaf flushing.

Leaf flush or bud weekly growth increment. The leaf flush or bud growth length increment in centimeters (cm) was measured and recorded.

Leaf Area (LA) per plant. The leaf area (LA) per plant was measured using the leaf area estimate employing a leaf weight basis in square centimeters. Leaf area measurements were done two weeks after leaf flushing or budding. The last leaf area measurement was used immediately before leaf harvest for feeding purposes. This was on the sixth week after pruning.

Shoot (Bolt or branch) length in centimeters. The bolt length is monitored weekly. Measurement was from the base of the bolt or bud to the tip of the terminal leaf. The final length of bolts or branches was measured immediately before leaf harvesting for feeding purposes.

Leaf herbage or biomass. The leaf fresh herbage or biomass was weighed in grams at leaf harvesting for feeding purposes.

Relative leaf growth rate (RGR). RGR calculated as the proportion of the increment in dry mass per increment in time and average biomass

The net assimilation rate (NAR). NAR is computed as the proportion of the biomass increment and leaf area.

Statistical Data Analysis

The data gathered were analyzed using a one-way analysis of variance for factorial Randomized Complete Block Design. Significant differences in treatment means were further subjected to paired treatment comparison using the Tukey's Honestly significant difference (HSD) test at a 5% level of significance. All statistical analysis was executed using Statistical Tool for Agricultural Research (STAR) 2.0 version.

Cultural Management and Practices

The general cultural management practices employed, such as weeding and irrigation, are undertaken weekly and as necessary.

III. RESULTS AND DISCUSSION

Effects of height pruning and gibberellic application to budding and shoot elongation

The individual effects of pruning and gibberellic concentration on the number of days to budding and soot elongation are shown in Table 1. Mulberry plants formed bud at heights of 0.75m and 0.50 meters with an average of 5.8 days after pruning, regardless of GA3 application. Independently, foliar spraying with 300 ppm GA3 promoted the earliest bud formation in 5.43 DAB, followed by 200 ppm and 100 ppm with budding periods of 5.43 and 6.00 DAB, respectively, compared to no GA3 application. In terms of budding time, no interaction effects were observed.

In terms of shoot formation and elongation, individual treatment effects were observed. The spraying of 200 ppm GA3 concentration exhibited the highest number of shoots on the first and sixth weeks of application.

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However, treatments employed manifested comparable shoot formation whether or not it is applied with different levels of GA3 or has undergone varying height pruning treatment. The application of GA3 significantly displayed elongation of shoots from the first and sixth week of plant regrowth from treatment application. The result conforms with Eltayb, Warag, & Elhiuri (2013) that pruning of *Morus sp*. at different heights has no significant effect on the plant's growth. The longest shoots were demonstrated in plants with 300 ppm GA3 and significantly highest among the other GA3 treatment applications. Applying 100 -200 ppm of GA3 to mulberry plants manifest comparable. This result conforms with the works of Atteya et al. (2018) that applying gibberellic acid improved branch length of primary and secondary branches of *Simmondsia chinensis*.

Table 1. The number of days to budding, shoot number, and shoot length (cm) as affected by pruning height and gibberellic acid application in 'Batac' mulberry variety

Treatment	DAB[1]	Shoot number[1]			Shoot length increment (cm)[3]		
		1st week	6th week	Increment	1st week	6th week	Increment
Pruning height							
P1-0.75m	5.85	33.20	46.13	10.52	6.18	110.75	104.55
P2-0.50m	5.88	35.62	105.02	8.62	6.20	105.02	98.84
Gibberellic acid application (Ga3)							
G0-0 ppm	7.00 d	28.07	38.57	10.50	3.84 *c*	38.73 *c*	80.89 c
G1-100ppm	6.00 c	31.50	37.43	8.93	6.00 *bc*	103.53 *bc*	97.53 *b*
G2-200ppm	5.43 b	41.00	52.70	11.70	6.20 *b*	109.50 *b*	103.30 *b*
G3-300ppm	5.03 a	37.07	47.20	10.13	8.72 *a*	133.77 *a*	125.05 a

[1]days to budding; [2]counted from sampled trees pruned; [3]measured as difference of the 1st and 6th weeks shoot length

Effects of height pruning and gibberellic application to leaf area and leaf area index

Figure 1 and Table 2 presents the mean leaf area (cm2) and leaf area index, respectively, of the different treatments affected by pruning heights. The first week demonstrated no significant difference in leaf area, but the sixth week manifested a substantial increase in mulberry plants pruned at 0.75 m however is statistically comparable with leaves obtained when pruned at 0.050 m height. A similar effect on leaf area was received when GA3 was applied independently. The 100 ppm application of GA3 extensively produced the highest leaf area on the 6th week after GA3 application with a mean of 28.42 cm2. Nonetheless, variations observed were comparable among all GA3 treatment applications.

On the other hand, the effect of various levels of gibberellic acid and height pruning employed on the leaf area index (LAI) revealed that the LAI increment of pruned plants at 0.50m is significantly higher than those pruned at 0.75m height. In addition, the LAI of mulberry plants applied with 300 ppm GA3 is considerably higher (0.5667) than those not applied with GA3 (0.4183).

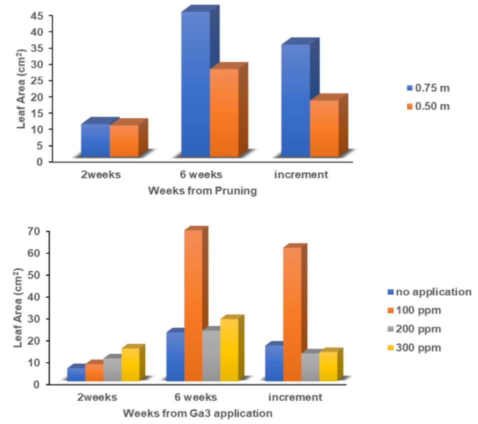

Figure 1. Leaf area (cm2) obtained from mulberry plants subjected to various height pruning techniques and levels of GA3 concentration.

Table 2. Leaf area index (LAI) obtained from mulberry plants two and six weeks after being subjected to different height pruning and levels of gibberellic acid (GA_3) concentrations

Treatment	Leaf area index (LAI)		
	2weeks	*Six weeks*	*Increment*
	Pruning height		
P1-0.75m	0.2008	0.4624	0.2633 b
P2-0.50m	0.1933	0.4858	0.2925 a
	Gibberellic acid application (GA3)		
G0-0 ppm	0.1217 *d*	0.4183 *c*	0.2967
G1-100ppm	0.1583 *c*	0.4483*bc*	0.2900
G2-200ppm	0.2083 *b*	0.4666 *b*	0.2583
G3-300ppm	0.3000 *a*	0.5667 *a*	0.2667
	P x G interaction		
P_1G_0	0.1333 *c*	0.4033 *c*	0.2703 *ab*
P_1G_1	0.1467 *c*	0.4633 *bc*	0.3167 *a*
P_1G_2	0.2033 *b*	0.4700 *bc*	0.2667 *ab*
P_1G_3	0.3200 *a*	0.5200 *b*	0.2000 *b*
P_2G_0	0.1100 *d*	0.4333 *c*	0.3233 *a*
P_2G_1	0.1700 *bc*	0.4333 *c*	0.2633 *ab*
P_2G_2	0.2133 *b*	0.4633 *bc*	0.2500 *ab*
P_2G_3	0.2800 *a*	0.6133 *a*	0.3333 *a*

The combined manipulation of leaf growth using the different levels of GA3 and different pruning heights manifested a significant contribution to LAI. Pruning the plants at 0.5 m applied with 300 ppm GA3 resulted in the highest LAI (0.6133) on its 6th week after application. LAI increment was observed at the same treatment with the highest manifestation of 0.3333 LAI, but similar to those plants undergoing 0.50m height pruning at different GA3 levels and plants experienced 0.75m height pruning combined with a maximum of 200 ppm GA3. The result of this study was further validated with Zhang et al. (2016) that GA3 increased vegetative growth, including leaf area, fresh weight, dry weight, chlorophyll content, the level of chlorophyll a and b. However, it inconsistently affects vegetative growth when applied in high bush berries, particularly in improving leaf area (Milić et al., 2018).

Corresponding author:dollente_armando46@yahoo.com

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Effects of height pruning and gibberellic application to leaf senescence and herbage yield

In terms of leaf senescence and herbage yield, similar responses were observed to mulberry plants pruned at various heights regardless of the application of GA3. On the other hand, the independent effect of spraying different levels of GA3 concentration to mulberry plants reflected significant effects to two parameters measured (Table 3) on the 4th and 6th weeks of treatment application. A faster rate of leaf senescence was observed in mulberry plants treated with 300 ppm, but it gave the highest yield, 447.5 g per plant. It is also significantly different in the biomass increment with 165.67 grams. The 0 GA3 application exhibited the least biomass increment. The result validated the study of several researchers that the application of GA3 improves dry matter yield accumulation in plants (Rohamare, Nikam, & Dhumal, 2013; Ghani et al., 2014; Miceli et al., 2019). The morphological and physiological traits were enhanced by GA3 treatments (biomass accumulation, leaf expansion, stomatal conductance, water use efficiency (WUE), Nitrogen use efficiency (NUE), with superimposable trends in both lettuce and rocket.

Table 3. Leaf senescence (L.) and biomass herbage yield (BH, grams (g) obtained from mulberry plants treated with different height pruning techniques and levels of GA_3 concentrations.

Treatment	Leaf Senescence (LS)		Biomass herbage (BH)		
	4 weeks	*6 weeks*	*4 weeks*	*6 weeks*	*Increment*
		Pruning height			
P1-0.75m	0.8259	1.0815	217.75	325.92	108.17
P2-0.50m	0.8318	1.1604	212.65	303.67	91.02
		Gibberellic acid application (GA3)			
G0-0 ppm	0.7071 *c*	0.7071 *c*	135.67 *c*	170.83 *d*	35.17 *b*
G1-100ppm	0.7071 *c*	0.7503 *c*	204.50 *b*	273.00 *c*	68.50*ab*
G2-200ppm	0.8553 *b*	1.2150 *b*	238.80*ab*	367.83 *b*	129.03*ab*
G3-300ppm	1.0459 *a*	1.8114 *a*	281.83 *a*	447.50 *a*	165.67*a*

Effects of height pruning and gibberellic application to leaf growth rate and net assimilation rate

Leaf growth rate and net assimilation rate as affected by pruning height are presented in Table 4. Statistical analysis showed no significant difference employed to the mulberry plants pruned at different heights. On an equivalent match, the net assimilation rate showed no significant difference among the pruning methods used.

Leaf growth rate significantly differed among the different GA3 concentrations. Plants treated with 300 ppm showed the highest rate, 11.83, which is highly significant to no GA3 application (2.51). The net assimilation rate was not significantly different among the GA3 concentrations. This conforms with the work of Kezemi (2014) on tomatoes. The application of gibberellic acid increased plant height, the number of branches, number of flowers per cluster, number of fruits per cluster, faster fruit growth, and increased fruit number, fruit firmness, weight, and yield.

Table 4. Relative leaf growth and net assimilation rate (g/m2/day) incurred by plants after being subjected to various pruning techniques and levels of GA3 concentration.

Treatment	Relative leaf growth rate[1]	Net assimilation rate[2]
	Pruning height	
P1-0.75m	7.7262	0.0021
P2-0.50m	6.5012	0.0018
	Gibberellic acid application	
G0-0 ppm	2.51 b	0.0010
G1-100ppm	4.89 ab	0.0012
G2-200ppm	9.22 ab	0.0028
G3-300ppm	11.83 a	0.0029

[1]*increment in dry mass per increment in time divided by average biomass;* [2]*computed as the proportion of the biomass increment and leaf area.*

Cost and Return Analysis. The most gaining among the treatments employed demonstrated pruning at 0.5 m with 300 ppm GA3 application depicting a net farm income of 3635.95, a benefit-cost ratio of 2.10, and an ROI of 109.81 % shown in Table 5. Pruning the plants at 0.5 m and no GA3 application manifested the highest loss by posting negligible profitability with a negative net farm income of -15.95, 0.99 benefit-cost ratios, and -0.66 return on investment. Mulberry plants treated with 300 ppm GA3 increase the silkworm rearing capability of the mulberry farm to at least 200 % through the increased mulberry leaf yield.

Table 5. Cost-Benefit analysis of utilizing a combination of pruning techniques and different levels of GA3 concentration to mulberry production.

Treatment	Yield (kg)[1]	Income (Php)	Operation Cost	NFI[2]	BCR[3]	ROI[4]
P_1G_0	910.00	2,730.00	2,411.00	319.00	1.13	13.23
P_1G_1	1,616.65	4,849.95	2,711.00	2,138.95	1.79	78.90
P_1G_2	1,811.65	5,434.95	3,011.00	2,423.95	1.80	80.47
P_1G_3	2,158.35	6,475.05	3,311.00	3,164.05	1.96	95.56
P_2G_0	798.35	2,395.05	2,411.00	(15.95)	0.99	(0.66)
P_2G_1	990.00	2,970.00	2,711.00	259.00	1.10	9.55
P_2G_2	1,866.65	5,599.95	3,011.00	2,588.95	1.86	85.98
P_2G_3	2,316.65	6,946.95	3,311.00	3,635.95	2.10	109.81

[1]*Based on the leaf biomass herbage multiplied by 5000 mulberry hills for ¼ hectare farm.*
[2] *NFI (Net Farm Income) = Gross Income- Total Maintenance Cost*
[3] *BCR -Benefit-Cost Ratio= Gross Income/Total Maintenance Cost*
[4]*Rate on Investment=(Gross income-Total Maintenance Cost)/Total Maintenance Cost*

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Figure 2. Crop stand of mulberry plants subjected to application of different pruning techniques and levels of GA3 concentration

Pruning is a vital cultivation practice that involves the methodical removal of branches from a plant to maintain high-quality produce. It is widely used in many plantation crops, including mulberry. Pruning is done in mulberry to adjust the leaf production period to synchronize with the leaf requirement for silkworm rearing and to extend the leaf production period seasonally (Noor et al., 2013). Pruning is an important cultural operation in mulberry cultivation because it improves the quality and quantity of leaf to maximize foliage utilization during rearing seasons. The effects of pruning heights on several parameters showed no significant difference, but there are substantial variations to a few variables. For example, the number of days to budding, number and length of shoots, leaf senescence, biomass herbage, leaf growth ratio, and net assimilation rate showed no significant difference. However, the leaf area of the mulberry pruned at 0.75 m gave a sizeable leaf area of 44.49. cm2 compared to 26.90 cm2 of mulberry plants pruned at 0.5 m. Studies have shown that the pruning method could stimulate stem growth and the number of twigs and leaves (ị Min, i Luong, & iu Hang, 2016). However, it may result in a smaller leaf size as compared with the leaf picking method. In India, tree density and pruning interval showed a prominent influence on forage and protein yields, whereas pruning height mainly affected the longevity and health of trees (Raj, Kunhamu & Kiroshima, 2015).

The outcome of the gibberellic acid concentrations on the leaf area per plant and leaf area index (LAI) showed significant differences among the treatments. Mulberry plants applied with 300 ppm gibberellic acid yielded effective extensive leaf area compared to other treatments, including the treatments with no GA3 application.

Gibberellic acid application stimulated increased biomass herbage production among the mulberry plants. However, mulberry leaf senescence is faster in the 300 ppm GA3 level, almost 50 % faster than the non-application of gibberellic acid. Therefore, the leaf biomass increment was primarily affected by GA3 concentrations.

The leaf growth ratio affected by gibberellic acid concentrations was accelerated most at 300 ppm, with the highest proportion of 11.83. The net assimilation rate, however, showed no significant difference in all the GA3 concentrations.

A study conducted in India revealed the influence of G3 on the growth, physiological and yield attributes of Stevia rebaudiana. It showed that at harvest (90 DAT), maximum internodal length and plant spread was recorded at G3 (500 ppm). The maximum leaf area and leaf area index was recorded at G2 (300 ppm). The use of plant hormones, specifically GA3, obviously improved the growth rate of alfalfa. Spraying of GA, especially with 200 mg/L, could effectively promote the height growth of alfalfa (Yang et al., 2012). In the study conducted by Garcia (2013), the physiologic properties of maize under the influence of IBA and GA3 application in 4-6 leaf and flowering stages were studied. Results indicated that using growth regulators in higher concentrations or implementing a combination of regulators are more effective. In the 4-6 leaf stage, the leaf area index, crop growth rate, and net assimilation rate are significantly affected by the application of growth regulators, which implies that these GRs have increased the power of source and thus increased the transport of assimilates to the sink including roots and young leaves.

Conclusion

As found and established under this experiment, the researcher accordingly concludes that the application of gibberellic acid on the mulberry plant after pruning either of the same height advances plant growth in terms of leaf augmentation and leaf deterioration. The higher concentrations produced the highest biomass herbage and yielded the highest mulberry foliage. However, the highest concentration enhances the fast yellowing of leaves or leaf senescence, tantamount to its speedy deterioration. There was no significant interaction effect between pruning heights and the different concentrations of GA3 applied to the mulberry plants. The highest profit was established by the highest concentration of GA3 with the highest net farm income, benefit-cost ratio, and rate of return per ¼ hectare mulberry farm.

Recommendation

The result of this study suggested that the application of 300 ppm of gibberellic acid to newly pruned mulberry plants either of any pruning height is an alternative option in increasing the leaf productivity of the mulberry plants, which turns out to be having higher returns in terms of net

Corresponding author:dollente_armando46@yahoo.com

profit as observed by higher indicators (BCR and ROI). Moreso, a follow-up study using the same variable or combining with other parameters should be regarded to validate the reliability of the results.

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Corresponding author:dollente_armando46@yahoo.com

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Corresponding author:dollente_armando46@yahoo.com