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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

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**ARTICLES**

**The effects of zinc (Zn) and C14-indoleacetic acid (IAA) on leaf senescence in *Helianthus annuus* L.**

Serap Sağlam-Çağ and Yener Okatan

**Interactive effects of virus and *Rhizobium* inocula on nodulation, growth and yield of cowpea**

L. B. Taiwo, M. A. Taiwo, S. A. Shoyinka, S. E. Jegede, J. A. Okogun, O. S. Oyatokun and G. G. Adebayo



## Full Length Research Paper

# The effects of zinc (Zn) and C<sup>14</sup>-indoleacetic acid (IAA) on leaf senescence in *Helianthus annuus* L.

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**Sequential leaf senescence is defined as a kind of programmed death events which is an important process in growth of plant. This study aimed to explore the sequential leaf senescence rate due to indoleacetic acid and lack of zinc (-Zn). Therefore, the effect of zinc and indole-3-acetic acid on senescence which occurs in *Helianthus annuus* (sunflower) cotyledons was analyzed. It was found that in cotyledons of seedlings grown in Hoagland solution which was prepared without addition of zinc senescence is delayed. It was recognised that in case of <sup>14</sup>C indoleacetic acid (IAA) which was given from apical tip not reaching the root and cotyledons, senescence does not occur in cotyledons. It was studied to get more information about physiological system of sequential leaf senescence.**

**Key words:** Sequential leaf senescence, cotyledon, zinc, <sup>14</sup>C indole-acetic acid (IAA), *Helianthus annuus* (sunflower).

## INTRODUCTION

Senescence is the final phase of plant vegetative and reproductive development, preceding the widespread death of cells and organs (Schmid et al., 1999; Guiboileau et al., 2010; Caswell and Salguero-Gomez, 2013). It has long been known that hormones regulate the progression of leaf senescence (Fletcher and Osborne, 1965; Misra and Biswal, 1980; Noodén and Leopold, 1988; Jibrán et al., 2013). In the process of senescence, destruction cases occur more than synthesis. From point of that view, definition of senescence is the process which increases destruction cases in cell and causes the plant to die.

The analysis made on leaf cells shows that during senescence consecutive metabolic events occur. These

events can be ordered as the synthesis of proteolytic enzyme (Colin and Thimann, 1972; Cheng and Kao, 1984; Hörtensteiner and Feller, 2002), the start of destruction of membrane proteins caused by these enzyme's activities, the decrease of quantity of protein and total nitrogen in the cell (Krul, 1974; Peterson and Huffaker, 1975; Peoples and Dalling, 1978, Prakash et al., 2001; Hopkins et al., 2007; Kaplan-Dalyan and Sağlam-Çağ, 2013), the acceleration of chlorophyll destruction (Peoples et al., 1980; Rodoni et al., 1997; Hörtensteiner, 2006; Darnel et al., 1990) and lipid destruction (Dhindsa et al., 1982; Harwood et al., 1982; Thompson et al., 1998; Hebel et al., 2008). It is accepted that transportation of nutrients in other leaves

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starting from the oldest organ to the youngest supports the nutrient drain hypothesis. According to another hypothesis called 'signal' hypothesis, a signal which is thought to be synthesized by developing seeds, is being transported to old leaves and causes senescence as a result of catabolic reactions. According to this hypothesis, if the signal center is eliminated, senescence does not occur (Lindoo and Noodén, 1977). But the above mentioned signal has been displayed that it could not be isolated (Moore, 1979; Ridge, 1991). It is obviously known that the cause of all biochemical events during senescence are related to gene expression (Draper, 1969; Sanders and Write, 1995; Hörtensteiner, 1997; Distelfeld et al., 2014). The meanings of these chemical events come into being only with the researches made by plant physiologists on plant's physiology. While it is being said that auxins (Wareing and Seth, 1967; Kahanak et al., 1978; Lim et al. 2003) delay senescence, researches made recently indicate that auxins (Palni et al., 1988; Lu et al., 2001; Gören and Sağlam-Çağ, 2007) accelerate the senescence. Otherwise, Noh and Amasino (Noh and Amasino, 1999) detected that auxin represses transcription of some genes whose expression is correlated with senescence.

## MATERIALS AND METHODS

*Helianthus annuus* L. seedlings were grown in intensity of 6000 lux light, under 12 h photoperiod and  $26 \pm 2^\circ\text{C}$ .

### Designation of senescence degree

To determine the senescence which occur in cotyledons of *H. annuus* quantitatively the method improved and used for soya bean's Anoca variety-show by Lindoo and Noodén (1976) was adapted to *H. annuus* cotyledons. For chlorophyll designation Arnon (1949) method was used. To determine total nitrogen quantity a method, formed with combination of Kjeldahl method and spectrophotometric measurement method was used (Lindoo and Noodén, 1976). The zinc quantity in the material was designated with atomic absorption spectrophotometer (AAS).

### Giving IAA to the cut ends of the decapitated seedlings

One to two days before senescence starts in cotyledons, seedlings were decapitated by being cut approximately 3 cm above the internodium cotyledons.  $10^{-5}$  M. IAA solution (treated) or water (control) was applied to decapitated surface.

### Giving $^{14}\text{C}$ -IAA to the seedlings on the top buds and nodium leaves

$10^{-5}$  mol.  $^{14}\text{C}$ -IAA (specific activity:40 mCi / mmol.) 1 drop 1% tween-20 was added per 1 ml. 60 and 120  $\mu\text{l}$  from IAA solution was dropped on plant's top bud, 80 and 320  $\mu\text{l}$  was dropped on nodium leaves. To hinder indolacetic acid's photo oxidation, this process was made when the plants were passing to dark period. The whole organ, of which radioactivity will be enumerated in  $\beta$  counter was

prepared accordingly. Counting value of the material per 5 min (cp5m=count per 5 min) was calculated.

### Statistic evaluation of the results

Standard deviation estimate was made to evaluate the results obtained from experiment and control groups statistically. In case of the number obtained when  $\pm$  values of differences between the result's square's total sum's square root is multiplied with three is found to be smaller than the difference between values, the difference is decided to be significant statistically.

## RESULTS

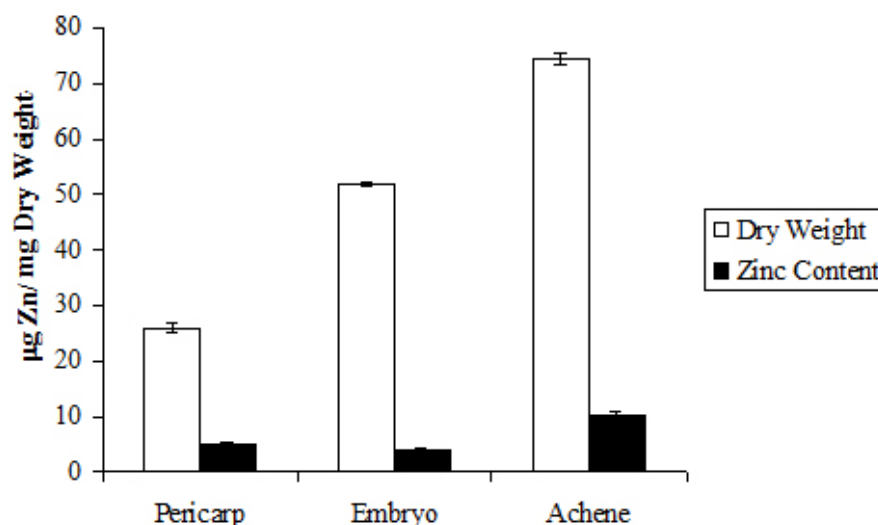
As it is known that zinc provides indole-acetic acid (IAA) stabilization and in case of zinc deficiency quantity of IAA decreases, this mineral's effects on plant growing and cotyledon senescence were analysed. The seedlings forming the experiment group were grown in Hoagland solution which does not include zinc and was diluted in 1/8 ratio (Table 1). Senescence delayed in cotyledons of seedlings in -Zn solution. Besides with the ingathering, when cotyledon senescence in plants grown with the existence of zinc (control) is 50% according to plastochron index (28<sup>th</sup> day), total chlorophyll and total nitrogen quantities in cotyledons of all seedlings belonging to experiment or control group (Table 2). The quantity of the zinc which is thought to exist in the seed naturally was measured with AAS (Figure 1). Nineteen days old *H. annuus* seedlings were divided into 4 groups. First group plants were intact (control). Other group plants were given IAA, NAA and H<sub>2</sub>O from truncated end being decapitated from under 2nd internodium. After this process the speed of senescence occurring in cotyledons was observed (Figure 2). In cotyledons of plants to which IAA and NAA applied senescence occurred quickly just like it occurs in cotyledons of intact plants. However, in a great majority of plants having a process with H<sub>2</sub>O, cotyledons remained green. Senescence did not occur in the cotyledons of the 17 days old experiment and control seedlings which were exposed to the same process and application. To determine first which organ as a target indolacetic acid after being produced in the plant is transported, 120  $\mu\text{l}$  from  $10^{-5}\text{M}$   $^{14}\text{C}$ -IAA+tween 20 upon top bud of the plants was dropped in. After dark period for 12 h, the quantity of  $^{14}\text{C}$ -IAA in the roots and cotyledons of the plants was stated (Table 3). Radioactivity existing in the root is found to be more than 20 times more than the radioactivity in cotyledons. From the values obtained it was understood that IAA given from top bud was transported quickly to the root. On the other hand, from the middle of the first internodium's of the seedlings, a part, approximately 1 cm was boiled with hot water vapour on the 17 day 60  $\mu\text{l}$   $10^{-5}$  M-IAA+tween 20 was dropped in the top bud of the plants of which cotyledons was just 100% green on the 32 day. After dark period for

**Table 1.** The effect of Zn (105 mg/L) on the green area (%) of the cotyledons from-day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Day	Average green area Percentage [Control (+ Zn)]	Day	Average green area Percentage [Control (- Zn)]
17	100.00 ± 0.00	20	100.00 ± 0.00
22	78.00 ± 0.26	23	73.93 ± 1.62
25	56.34 ± 1.42	27	49.45 ± 1.23
29	32.73 ± 2.46	30	21.61 ± 1.72
33	00.00 ± 0.00	34	00.00 ± 0.00

**Table 2.** The effect of Zn (105 mg/L) on the chlorophyll and nitrogen content of the cotyledons from 28 day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Hoagland	mg N / g cotyledon	%	mg chlorophyll / cotyledon	%
Hoagland (- Zn)	129.603 ± 4.256	100	0.0449 ± 0.005	100
Hoagland (+ Zn)	108.109 ± 2.562	83.4	0.0382 ± 0.002	74.2

**Figure 1.** Dry weight and Zn quantity in pericarp, embryo and achene.

12 h, radioactivity in different organs of the plants was measured (Table 4).

As the radioactivity difference counted in organs under the 1 internodium's boiled part was insignificant statistically, it was understood that radioactivity can pass downwards from boiled area in trace quantity. Besides, although it was found on boiled part, on the leaves of the 2 internodium a statistically significant quantity of radioactivity could not be found. It was stated that radioactivity had accumulated in a great quantity on the boiled part of internodium.

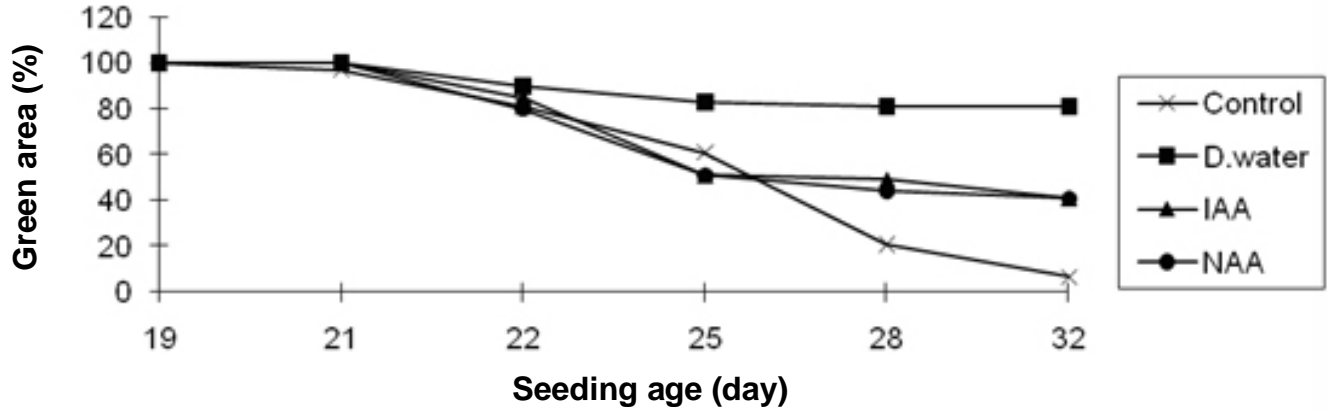
To the first leaves (second nodium leaf) after cotyledon of 19 days old plants totally 80µl <sup>14</sup>C-IAA, 40 per each, was applied. After plants being ingathered on different

days (Avery et al., 1937; Lindoo and Noodén, 1976; Papadopoulus et al., 1985; Noodén and Leopold, 1988), radioactivity on root and 3. nodium leaves was measured (Table 5).

It was recognised that <sup>14</sup>C-IAA applied to 2 nodium leaves was transported to root first and then from there by xylem, was transported to the leaves making transpiration.

## DISCUSSION

In this research, the effects of zinc and indolacetic acid, which is a growing hormone, on senescence was



**Figure 2.** Green area (%) of cotyledons of 19 days old *H. annuus* seedlings which were decapitated below the second internode and treated with  $10^{-5}$ M IAA,  $10^{-5}$ M NAA or  $H_2O$  from the truncated end.

**Table 3.**  $^{14}C$  amounts in the roots and cotyledons of *H. annuus* seedlings treated with  $10^{-5}$  M  $^{14}C$ -IAA (\* Significant).

Organ	Count / 5 min	Radiation of background	Difference
Cotyledon	299.85 ± 31.25	288.36 ± 3.015	11.49
Root	875.41 ± 29.09	288.36 ± 3.015	587.05 *

**Table 4.**  $^{14}C$  amounts in different organs of *H. annuus* seedlings treated with  $10^{-5}$  M  $^{14}C$ -IAA (\* Significant).

Organ	Count / 5 min	Radiation of background	Difference
2nd node leaves	306.92 ± 7.05	288.36 ± 3.015	18.56
First internode	1039.33 ± 57.63	288.36 ± 3.015	750.97 *
100% Green cotyledon	299.87 ± 7.66	288.36 ± 3.015	11.51
Hypocotyl	304.00 ± 11.82	288.36 ± 3.015	15.64
Root	313.00 ± 10.64	288.36 ± 3.015	24.64

**Table 5.**  $^{14}C$  amounts in the roots and 3<sup>rd</sup> node leaf of *H. annuus* seedlings leaf on different days after  $10^{-5}$  M  $^{14}C$ -IAA treatments on the 2<sup>nd</sup> nodium. (\* Significant).

Day	Root (Count / 5 min)	3rd nodium leaf (count / 5 min)	Radiation of background	Difference (root)	Difference (3 rd nodium leaf)
20	318,44 ± 11.627	302,67 ± 3.567	288.36 ± 3.015	30.08	14.31 *
21	489,11 ± 34.310	352,22 ± 10.97	288.36 ± 3.015	200.75*	63.86 *
22	454.00 ± 45.287	342.00 ± 14.09	288.36 ± 3.015	165.64 *	53.64 *
25	485,67 ± 11.794	712,89 ± 131.27	288.36 ± 3.015	197.31 *	424.53 *

searched. In a reseach (Ray and Choudhuri, 1981), it was supported that hormones (IAA, GA, Kinetin) plays the most important role in transporting nutrients to seeds that develops as an endogenic hormone resource. It is known that the deficiency of the hormones which perevents senescence (for ex: cytokinin) may cause senescence. Palni et al. (1988) mentions that auxin has

an effect on cytokinin's metabolism and this effect is actualized by oxidase enzyme. While some researchers (Jacobs and Cready, 1967; Sanchez-Bravo et al., 1991) declare that indolacedic acid localize in cortex, vascular tissue and pith, auxin is transported in vascular and epidermal tissues, other researchers (Bangerth, 1994; Eköf et al., 1995; Li et al., 1995; Shimizu-Sato et al., 2009)

emphasized that intact plants's cytokinins in xylem exudate are under the control of polar auxin transportation system. Hare and Staden (1994) expressed that cytokinin catabolism which becomes true with the activity of sitokinin, a specific enzyme oksidase realizes death in plant tissue and moreover stated that auxin plays the role of allosteric systematizer increasing this enzyme's activity.

It is known that indolacetic acid is synthesized in the end of stem and zinc provides the stabilization of indolacetic acid (Skoog, 1940; Takaki and Kushizaki, 1970). In the early development phase of the plants of which endogen IAA quantity was decreased by being grown in zinc deficiency, IAA that is under the control of the quantity of zinc in the seed has such a quantity that it delays the senescence but can provide growing. But zinc which is given with Hoagland solution in addition to the zinc quantity in the seedling may be impulsive in senescence or may delay growing because of its toxic effects on some enzyme systems related to growing. Likewise, Sağlam-Çağ and others (Sağlam-Çağ et al., 2004) emphasized that senescence was delayed in excised cotyledons in the solution lacking zinc. In that research, in the existence or deficiency of zinc, IAA which can be controlled endogenously was held to be responsible for the change in senescence's speed. In some experiments which  $^{14}\text{C}$ -IAA was used (Hew et al., 1967), it was noticed that IAA given from truncated end of the stem goes through stem axis quickly and don't enter to leaves. Also, in this research it was found that  $^{14}\text{C}$ -IAA was transported to root without touching at leaves. Moreover, as  $^{14}\text{C}$ 's internodium does not goes through boiled part, it was noticed that it could not reach the root and cotyledons and senescence didn't occur in cotyledons.

We can assert that sequential leaf senescence is related to the occurrence of metaxylem after protoxylem and in this event, with IAA's effect on xylem formation, it may come on the scene. Just before senescence, although senescence occurred when IAA was applied from truncated top, cotyledons remained green when IAA was applied in early phase. Researchers (Shimomura et al., 1988; Jones et al., 1989; Jones, 1994) indicated that there are 2 different receptors in plasma membrane connecting IAA and in recent years it was determined that first one of these receptors isolated is related to cell growing but then any absolute information about second IAA receptor's function wasn't given (Darnel et al., 1990; Cooper, 1997).

As a result of our research, we saw that zinc, providing IAA stabilization accelerates senescence; in the researches made with  $^{14}\text{C}$ , as its internodium does not go through boiled part  $^{14}\text{C}$ -IAA given from apex, it can not reach root and cotyledons and senescence does not occur in cotyledons. It became certain that it was transported to the root without touching at leaves and this transportation is made by parenchymatic living tissues not xylem. This research indicated that senescence signal may be indoleacetic acid or a substance like indoleacetic acid.

## Conflict of interests

The author(s) have not declared any conflict of interests.

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## Full Length Research Paper

## Interactive effects of virus and *Rhizobium* inocula on nodulation, growth and yield of cowpea

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The effect of separately inoculating cowpea cultivars, 'Ife brown' (IF) and "Owode" (OW) with *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea yellow mosaic virus* (CYMV) and *Rhizobium* IRc 284(RH-284) on nodulation was investigated. Also, the effect of inoculating the cowpea cultivars with RH-284 on the severity of infection caused by the viruses was studied. The interactive effects of inoculating cowpea with RH-284 and each of CABMV, CYMV, *Southern bean mosaic virus* (SBMV) and *Blackeye cowpea mosaic virus* (BICMV) on nodulation, growth and yield of IF and OW were also investigated. The results showed that infection by CYMV and CABMV significantly reduced nodulation by about 20-30% and 40-45% in IF and OW, respectively. Inoculating with RH-284 alone significantly increased nodulation by about 20% in both cowpea cultivars. In the interactive study involving virus-RH 284 inocula, slight but non-significant increases of 22, 2 and 9% in nodule number were observed in IF inoculated with RH-284 and SBMV, CYMV and CABMV, respectively. The differences observed in the nodule, shoot and seed weights were not significantly different from those of the control. There was a negative correlation between nodule number and severity of symptom. BICMV caused the most severe effect on the two cowpea cultivars. It reduced the number of nodules by 55-66% with or without RH-284. It also caused significant reductions of over 80% in nodule and seed weights of OW. In conclusion, increase in nodulation reduced viral disease severity, the slight but non-significant increases observed in the growth and yield parameters suggest that improved nodulation can be advantageous to cowpea.

**Key words:** Blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, cowpea yellow mosaic virus, southern bean mosaic virus.

### INTRODUCTION

Cowpea is an important food and fodder legume in the sub-humid tropics of Africa. As a food, the grain is an important source of dietary protein especially for the West African populace where two-thirds of the world's cowpea grain is produced. The crop has therefore attracted a lot of attention from researchers who have in recent years intensified their efforts at improving its agronomic and

nutritional qualities (Rachie, 1985).

Cowpea grain yields vary greatly in different parts of the world. Singh (1980) estimated that the average yield for the crop grown in monoculture is about 1.5 t/ha in the United States of America, 650 kg/ha in South America and Asia, and is often below 400 kg/ha in Africa. The low cowpea yield in Africa is mainly due to pests and

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diseases. Among the numerous pathogens the effect of these viruses can be devastating and they are a major constraint to increased grain production. Although, nine viruses are reported on cowpeas in sub-Saharan Africa (Taiwo, 2003), only *Cowpea aphid-borne mosaic virus* (CABMV) genus *Potyvirus* and *Cowpea yellow mosaic virus* (CYMV) genus *Comovirus* are considered to be very important as far as geographical distribution, pathogenic variability and yield losses are concerned (Thottappilly and Rossel, 1992). *Cowpea mottle virus* (CMeV) genus *Carmovirus*, *Southern bean mosaic* (SBMV) genus *Sobemovirus*, *Cowpea golden mosaic* (CGMV) genus *Begomovirus*, *Blackeye cowpea mosaic virus* (BICMV) genus *Potyvirus* and *Cucumber mosaic viruses* (CMV) genus *Cucumovirus* are considered to be of localized importance (Taiwo, 2003).

Yield reduction attributable to CYMV infections range from 40-100% (Chant, 1960; Wells and Deba, 1961; Shoyinka, 1974; Gilmer et al., 1974) while a virus suspected to be CABMV caused a complete loss in yield in northern Nigeria (Raheja and Leleji, 1974).

Efforts to control viral diseases of cowpea may involve one or more measures intended to reduce sources of infection, roguing of diseased plants and the use of insecticides to prevent virus transmission. Currently, host plant resistance is the most effective method of controlling cowpea viruses in Africa (Thottappilly and Rossel, 1992).

However, like many other legumes, cowpea can symbiose with nodule bacteria present in most tropical soils. Effective cowpea-*Rhizobium* symbiosis fixes more than 150 kg/ha of N<sub>2</sub> and supply a considerable amount of the N<sub>2</sub> requirement of plants (Summerfield et al., 1977). This attribute allows healthy growth and hence optimum yields. Symbiotic N<sub>2</sub> fixation in cowpea-root nodules is a result of the complex biological and biochemical interactions between the host legume and the rhizobial endophyte. If the process is efficient, the plant grows healthily, thereby minimizing the impact of diseases. It is therefore, expected that the extraneous application of *Rhizobium* to cowpea at planting should increase its population in the soil, ensure optimal and rapid growth of the crop, and thus enhance its resistance to phytopathogenic agents, especially viruses.

This study was therefore carried out to assess the effect of rhizobial inoculant on growth and seed yield of cowpea, the impact of viral infection on nodulation, vegetative and seed yield of the crop and determine the extent of amelioration of the impact of viral infection on cowpea by rhizobial inoculant.

## MATERIALS AND METHODS

Two greenhouse studies were carried out to determine the effect of inoculating 2 cowpea (*Vigna unguiculata* L. Walp) genotypes (Ife Brown (IF) and Owode (OW)) with cowpea rhizobium IRc 284 (RH-284) and virus. The interaction of the microorganisms on the 2 cowpea genotypes was also evaluated.

### Preliminary greenhouse 1

It was a factorial experiment consisting of 2 cowpea genotypes (IF and OW, one rhizobial inoculant (RH-284) I+ and I<sub>0</sub> and 2 virus strains, CABMV and CYMV, and control. Thus 12 treatment combinations were obtained. The treatments were replicated 3 times and arranged in a randomized complete block design (RCBD). In this study, only nodule number was assessed.

### Greenhouse 2

In this experiment, a more detailed study was carried out using the 2 genotypes in the preliminary work as well as rhizobium strain. However, additional viruses, BICMV as well as SBMV were used. Therefore, a factorial design that consisted of 2 cowpea genotypes, 2 rhizobial inoculants I+ and I<sub>0</sub> and 4 virus strains with a control was set up. The twenty treatment combinations obtained were replicated 8 times.

### Rhizobial inoculation

Seeds of IF and OW were inoculated with the cowpea *Rhizobium* IRc 284 (RH-284) (obtained from the culture bank of the International Institute of Tropical Agriculture (IITA)) at a concentration of 10<sup>7</sup> - 10<sup>8</sup> colony forming unit (CFU) before planting in soil at three seeds per pot.

The seeds for the control plants were not inoculated with the rhizobial inoculum. The RH-284 inoculum was prepared in yeast extract mannitol broth (YEM) (Vincent, 1970), (Mannitol, 20.0 g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5g; yeast extract, 0.2 g; FeCl<sub>3</sub>, 2.0 mg; MnSO<sub>4</sub>.H<sub>2</sub>O, 4.0 mg; in 1 L of distilled water pH 6.8). The medium (100 ml/flask) was autoclaved at 121°C for 15 min, cooled, inoculated with RH-284 and incubated for about 7 days on a rotary shaker at 28°C, before being used as inoculum. The number of colony forming units (CFU) on plate count agar was 10<sup>8</sup>. Numbers of nodules on 3 plants of the replicates each of RH-284 inoculated and un-inoculated plants were determined after six weeks. A photograph of nodules on roots was also taken.

### Virus inoculation

Three seeds were separately sown per pot. The seedlings were later thinned to 2. Seedlings of 8-day old plants of IF OW that were to be inoculated were mechanically inoculated with the viruses. Mechanical transmission was with viral inocula prepared in 0.01 M phosphate buffer pH 7.1 according to Nordam (1973), while buffer inoculated plants served as control. The inoculated plants were labeled and kept in an insect-proof screen house at temperatures of 28-32°C. They were regularly observed for symptom development. The severity of symptoms observed was rated on a scale of 1 - 5, (5 for very severe infection, sometime death, 4 for severe infection, 3 for moderate infection; 2 for mild infection and 1 for very mild infection).

Plants were harvested after six weeks, the plants were carefully uprooted, the soil was washed off and the nodules on the roots counted.

Nodule number and weights, vegetative weights were determined on 3 of the 8 replicated plants while seed yield was determined on the other replicates at maturity.

Analysis of variance (ANOVA) was used to determine significant differences and means of the significantly different sources were separated using Duncan multiple range test.



**Table 1.** Analysis of variance table for nodule number in preliminary Experiment.

Variation	df	Mean sum of square
Cowpea (block)	1	0.798
Virus	4	16.69**
Inoculant (RH)	1	39.33**
Cowpea x RH	1	0.090
Cowpea x Virus	4	0.040
Virus x RH	4	1.990
Cowpea x Virus x RH	4	0.356
Error	24	5.267

\*\* Significant at  $P \leq 0.01$ .

**Table 2.** Effect of viral and rhizobial inoculation on nodulation in the preliminary study.

	Treatment	Nodule number
Virus inoculant treatments	control	5.83 <sup>a</sup>
	CYMV	4.35 <sup>b</sup>
	CABMV	3.87 <sup>b</sup>
Rhizobia inoculant treatments	control	3.78 <sup>b</sup>
	RH-284	5.59 <sup>a</sup>
	Standard error	0.34

Means followed by same letter in a column in each treatment are not significantly different.

**Figure 1.** Effect of virus on nodulation in roots of A: healthy and B: CABMV = Cowpea aphid-borne mosaic virus-infected cowpea plants.

## RESULTS

Analysis of variance in Table 1 showed that the 2 viruses had significant ( $p < 0.01$ ) effect on number of nodules in the 2 cowpea genotypes in the preliminary study. Rhizobium inoculant significantly impacted on the number of nodules. The number of nodules on Ife Brown (IF) and Owode (OW) cowpeas was not significantly different (Table 2). Cowpea genotypes without virus had higher number of nodules when compared with virus infected plant. The number of nodules was significantly higher ( $p < 0.01$ ) than that of plants inoculated with CYMV and CABMV by over 20 and 30%, respectively. Number of nodules on plant inoculated with CABMV and CYMV were not significantly different. Rhizobium significantly ( $p < 0.01$ ) increased nodule number by 30% when compared with uninoculated plant.

Also, in the preliminary study, virus infection adversely affected the number and size of the nodules in the cowpea genotypes. Fewer and smaller nodules were formed and the growths of root hairs as well as the lateral roots were impaired in virus-infected plants (Figure 1).

In the second greenhouse study, nodule numbers were not significantly different from each other in the 2 cowpea genotypes but vary significantly with respect to nodule weight in the analysis of variance (Table 3). The viruses impacted significantly on nodule number and weights. There were also significant ( $p < 0.01$ ) cowpea-virus interaction as well as virus-rhizobium interaction on nodule weight. The 3 factors also interacted on the number of nodules (Table 3).

Root weights in the 2 cowpea genotypes varied significantly from each other but not with the shoot weight (Table 4). There was significant interaction of cowpea and virus on root while virus and rhizobium interaction significantly impacted on weight of shoot. Viruses as well as rhizobium had significant effect on weight of seeds.

Table 5 shows the effect of variety, rhizobium and virus on nodulation, vegetative and seed yield of cowpea. The number of nodules in the 2 cowpea genotypes did not differ significantly but the weight of nodules, root and shoot in Ife Brown were significantly higher than that of Owode variety. Owode genotype however, gave a significantly higher seed yield than IF. Rhizobium significantly increased nodule number but not nodule weight. The nodule weights were also significantly increased by RH-284. Numbers of nodules were significantly reduced by BICMV (IT16) when compared with other viruses and control. Nodule weight in BICMV was significantly reduced by 50% relative to virus-free control. Nodule weight in BICMV-treated cowpea was 30% lower than control and SBMV-treated plants. Weight of shoot and seed were also significantly reduced by 60 and 50% respectively by BICMV relative to control. Seed weights of cowpeas inoculated with CYMV and CABMV were significantly reduced relative to control.

The interactive effect of the 3 factors, namely; variety, rhizobium, rhizobium and viruses on the parameters are

**Table 3.** Analysis of variance table for nodule number and weight in green house (Experiment 2).

Variation	df	Mean sum of square (number of nodule)	Mean sum of square (weight of nodule)
Cowpea (Block)	1	0.86	2.32**
V	4	129.84**	9.81**
Innoculation (RH)	1	17.7	0.26
Cowpea x RH	1	0.63	0.08
Cowpea x Virus	4	4.11	0.92**
Virus x RH	4	8.14	1.12**
Cowpea x Virus x RH	4	22.41*	0.48
Error	24	10.4167	0.016111

\*\* Significant at  $P \leq 0.01$ , ANOVA; \* Significant at  $P \leq 0.01$ , ANOVA

**Table 4.** Analysis of variance table for weight of shoot and seeds greenhouse (experiment 2).

Variation	df	Shoot weight			Weight of roots		
		Sum of square	Mean sum of square	F	Sum of square	Mean sum of square	F
Cowpea (Block)	1	0.822	0.822	0.32	2.48	2.48	17.6**
V	4	63.84	15.96	6.213**	29.96	7.49	53.1**
Innoculation (RH)	1	0.54	0.543	0.21	0.01	0.01	0.07
Cowpea x RH	1	2.426	2.426	0.95	0.01	0.01	0.07
Cowpea X Virus	4	15.315	3.829	1.491	0.83	0.21	1.5
Virus X RH	4	41.581	10.395	4.05	0.73	0.18	1.3
Cowpea X Virus X RH	4	3.785	<u>0.946</u>	<u>0.37</u>	1.11	<u>0.28</u>	1.987
Error	24	61.65	2.56875		3.3816	0.1409	

\*\*Significant at  $P \leq 0.01$ , ANOVA, \* Significant at  $P \leq 0.01$ , ANOVA. V, Virus; RH, rhizobium; Cowp, cowpea and RH, rhizobium.

**Table 5.** Effects of varietal difference, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Genotypes cowpea	Nodule number	Nodule weight (g)	Weight of sht (g)	Weight of seeds (g)
IF	8.29 <sup>a</sup>	0.36 <sup>a</sup>	2.61 <sup>a</sup>	2.44 <sup>b</sup>
OW	8.09 <sup>a</sup>	0.33 <sup>b</sup>	2.40 <sup>a</sup>	2.80 <sup>a</sup>
<b>Rhizobial inoculant</b>				
RH <sub>0</sub>	7.72 <sup>b</sup>	0.35 <sup>a</sup>	2.42 <sup>a</sup>	2.61 <sup>a</sup>
RH	8.66 <sup>a</sup>	0.39 <sup>a</sup>	2.59 <sup>a</sup>	2.62 <sup>a</sup>
<b>Virus inoculant</b>				
V <sub>0</sub>	9.88 <sup>a</sup>	0.39 <sup>a</sup>	2.77 <sup>ab</sup>	3.10 <sup>a</sup>
CYMV	9.36 <sup>a</sup>	0.37 <sup>b</sup>	2.31 <sup>b</sup>	2.83 <sup>b</sup>
CABMV	8.75 <sup>a</sup>	0.37 <sup>ab</sup>	2.68 <sup>ab</sup>	2.78 <sup>b</sup>
BICMV(IT16)	3.16 <sup>b</sup>	0.21 <sup>c</sup>	1.00 <sup>c</sup>	1.41 <sup>c</sup>
SBMV-Oyo	9.81 <sup>a</sup>	0.38 <sup>ab</sup>	3.77 <sup>a</sup>	2.95 <sup>ab</sup>
EMS	4.32	0.20	2.54	0.14

CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium, IF = lfe brown and OW = Owode

found in Table 6. Only dry weight of shoot of IF that was free of virus was significantly ( $p, 0.01$ ) increased by RH-284. Number of nodules as well as weight of seeds of IF inoculated with CYMV was significantly enhanced by rhizobium RH-284. With CABMV on IF, dry weight of

shoot was significantly increased by rhizobial inoculant. Even though lfe brown cowpea was infected with BICMV, the number of nodules was increased by RH-284. Number of nodules, weight of shoot and seeds of IF were significantly increased by RH-284 relative to un-inoculated

**Table 6.** Interactive effects of variety, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Treatments	Nodule number	Nodule weight	Weight of shoot	Weight of seed
1. IFV <sub>0</sub> RH	11.32 <sup>abc</sup>	0.4 <sup>abc</sup>	4.33 <sup>ab</sup>	2.49 <sup>ef</sup>
2. IFV <sub>0</sub> RH0	8.98 <sup>bcdef</sup>	0.20 <sup>cd</sup>	3.52 <sup>efg</sup>	2.64 <sup>cdef</sup>
3. IFCYMRH	7.62 <sup>ef</sup>	0.30 <sup>bcd</sup>	3.46 <sup>efg</sup>	2.56 <sup>def</sup>
4. IFCYMV	3.15 <sup>g</sup>	0.11 <sup>d</sup>	2.91 <sup>g</sup>	1.43 <sup>g</sup>
5. IFCABMRH	7.60 <sup>ef</sup>	0.2 <sup>cd</sup>	3.99 <sup>abcde</sup>	3.00 <sup>abcde</sup>
6. IFCABMV	6.09 <sup>gf</sup>	0.2 <sup>cd</sup>	3.43 <sup>efg</sup>	3.43 <sup>a</sup>
7. IFBICMRH	10.81 <sup>abcd</sup>	0.40 <sup>abc</sup>	4.19 <sup>abcd</sup>	2.86 <sup>bcdef</sup>
8. IFBICMV	8.04 <sup>def</sup>	0.28 <sup>bcd</sup>	3.58 <sup>def</sup>	3.14 <sup>abc</sup>
9. IFSBMRH	3.16 <sup>g</sup>	0.12 <sup>d</sup>	2.12 <sup>h</sup>	1.43 <sup>g</sup>
10. IFSBMV	10.43 <sup>abcde</sup>	0.27 <sup>bcd</sup>	3.51 <sup>efg</sup>	3.13 <sup>abc</sup>
11. OWV0RH	10.27 <sup>abcde</sup>	0.33 <sup>abc</sup>	4.43 <sup>a</sup>	3.25 <sup>ab</sup>
12. OWV0	8.85 <sup>cdef</sup>	0.17 <sup>cd</sup>	3.41 <sup>efg</sup>	2.64 <sup>cdef</sup>
13. OWCYMRH	9.62 <sup>bcde</sup>	0.28 <sup>bcd</sup>	4.24 <sup>abc</sup>	2.40 <sup>f</sup>
14. OWCYMV	4.14 <sup>g</sup>	0.10 <sup>d</sup>	1.56 <sup>h</sup>	1.41 <sup>g</sup>
15. OWCABMRH	12.37 <sup>a</sup>	0.55 <sup>a</sup>	4.33 <sup>ab</sup>	2.60 <sup>def</sup>
16. OWCABMV	11.85 <sup>ab</sup>	0.19 <sup>cd</sup>	3.78 <sup>bcdef</sup>	3.25 <sup>ab</sup>
17. OWBICMRH	8.80 <sup>cdef</sup>	0.17 <sup>cd</sup>	3.55 <sup>efg</sup>	3.19 <sup>ab</sup>
18. OWBICMV	9.69 <sup>abcde</sup>	0.16 <sup>cd</sup>	3.62 <sup>cdef</sup>	3.03 <sup>abcde</sup>
19. OWSBMRH	3.17 <sup>g</sup>	0.10 <sup>d</sup>	1.67 <sup>h</sup>	1.41 <sup>g</sup>
20. OWSBMV	8.85 <sup>cdef</sup>	0.5 <sup>ab</sup>	3.32 <sup>fg</sup>	3.06 <sup>abcde</sup>

Standard error of mean. All values are averages of 4 replicates. Values followed by the same letters of the alphabet are not significant. CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium.

plant carrying SBMV.

On virus-free Owode genotype, dry weight of shoot and seed were significantly increased by RH-284 relative to uninoculated plant. However with CYMV, number of nodules, dry weight of shoot and seed were significantly increased relative to RH-284 free plant. Weight of shoot and seeds as well as nodule weight of CABMV-inoculated plant were significantly enhanced by rhizobium. Rhizobial inoculation had no positive effect on all the parameters of BICMV inoculated OW genotype but enhanced nodule number and weight as well as weight of shoot and seeds of SBMV inoculated OW.

## DISCUSSION

Yield reduction or outright crop failure in cowpea resulting from viral attack are of common occurrence in sub-Saharan African. This has necessitated the need to carry out studies on biological strategy to reduce the incidence of cowpea viruses.

Results in the preliminary study have shown that the 2 viruses used had significant negative impact on the number of nodules in the 2 cowpea genotypes used. While no significant difference was observed in the 2 cowpea genotypes used with respect to their growth. Cowpea yellow mosaic virus (CYMV) and cowpea aphid

borne mosaic virus (CABMV) significantly depressed nodule number. Infection of cowpea by these viruses led to impairment of vegetative growth with most of the leaves curling. This indicates that some physiological processes of growth might have been impaired. Nodulation, according to Denarie et al. (1996) and Spaink (2000) is a physiological process that involves the production of diffusible plant and bacterial metabolites such as flavonoids and lipo-chitooligosaccharides respectively, which trigger certain steps of the processes. It is assumed that interference of these processes had caused the reduction in nodule number. Effective nodulation also depends on the population of infective rhizobia in soils. Increasing the soil population through inoculation in this study had led to improved nodulation.

Significant increases in nodule number and root weight were observed in *Rhizobium* inoculated cowpeas. Legume inoculation is a process for the manipulation of rhizobial microflora for improving crop productivity and soil fertility (Keyser and Li, 1992). Although, rhizobial species are as widely distributed as the legumes themselves, there are many soils where suitable strains are absent, or where the population density is as low as to pose a threat to legume establishment and effective nodulation and N<sub>2</sub> fixation. The population of indigenous rhizobial in most tropical soils is very low and these indigenous strains are less efficient in fixing nitrogen

(Ahmad et al., 1981; Ahmad and Mclaughling, 1985). The selection and application of specific rhizobial inoculant as carried out in this study can be exploited in sustainable cowpea production. It was generally observed that nodule number and other growth parameters assessed were significantly reduced by BICMV (IT16). The infection caused by BICMV demonstrated this by resulting in significant growth and yield reductions in the cowpea cultivars used. This confirms previous results by Owolabi et al. (1988) which indicated that BICMV posed a more serious threat than any other virus to the production of cowpea cultivars. This was shown in more severe symptoms including death of plants at an early age in BICMV infections. Viruses such as CABMV and CYMV do cause severe symptoms including mosaic, green vein-banding, stunting and dramatic yield losses. The loss may range between 40-100% depending on the age of the plant at the time of infection (Chant, 1960; Raheja and Leleji, 1974). These results agree with those reported by Tu et al. (1970) and Hair and Miller (1982) working with clover and cowpeas, respectively. Patil and Sayyad (1994) reported a greater reduction in nodule number in virus infected cowpea than in *Rhizobium*-virus infected plants. They also reported that the reduction in the fresh weight of the nodules was greater than the reduction in fresh weight of plant due to CYMV.

On the interactive effect of RH-284 and viruses, inoculation of IF and OW with RH-284 significantly increased some parameters in spite of viral infection. This implies that virus infected plants produced more severe symptom of infection when they were not inoculated with rhizobium than *Rhizobium*-virus treated plants. The increase in nodule number and some other parameters of RH-284 inoculated plant relative to un-inoculated control indicated that the inoculant strain contributed significantly to nodulation. Eaglesham (1985) noted that it might be safer to rely on effective inoculant strain than breed plant for the ability to nodulate with indigenous strains of unknown potential. However, rhizobial inoculant can only be successful if it is more competitive than the native rhizobial in nodule formation and N<sub>2</sub> fixation. Generally, in legume symbiosis, regulation of N<sub>2</sub>-fixation is mediated by the host legume rather than by the bacterial symbiont (Giller and Wilson, 1993). In this study, infection by the viruses impaired some growth and yield parameters in spite of rhizobial inoculation. The inference drawn here is that it is only the severity of infection that can be reduced, infection cannot be stopped with rhizobium inoculation and not the infection of virus itself. The actual effect of increased nodulation and N<sub>2</sub>-fixation was subsumed in the negative impact of virus attack, leading eventually to the complete suppression of the growth advantage conferred on the plants by the rhizobial inoculants.

There may be the need to experiment with various concentrations of the rhizobial inoculant, in order to derive maximum advantage from the positive impact of

rhizobial inoculation.

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