MUNGBEAN RESIDUE EFFECTS ON THE GROWTH PARAMETERS OF A SUCCEEDING MUNGBEAN CROP

by

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ABSTRACT

Investigations of mungbean *(Vigna radiata* (L.) Wilczek) residues, which have been found to have adverse effects and cause substantial yield reduction on subsequent mungbean crops grown in rapid rotation, were undertaken to determine the source of phytotoxin and study its effects on growth parameters.

There was no effect on plants grown in pots of steam-sterilized soil that had received leachates from actively growing mungbean plants in sand culture, or in pots that had received leachates of roots and leaves decomposing in sand.

Effects from residues of previous mungbean crops were demonstrated when succeeding mungbean crops were grown such that their roots were in direct physical contact with the residue. Plants grown in soil in which root-leaf residue mix had been incubated for one week prior to seeding were about 50% of control in total dry weight at any sampling date. Total dry weight was further reduced to about 40% when incubation time was increased to three weeks. Separate experiments with root and leaf residues showed that leaf residues were about 12.3% more toxic than root residues on a proportionate residue weight basis. The combination of leaf and root residues did not show additive effects.

Incorporation of the residues into the soil prevented normal seedling development. Plants growing from residue-treated soil had more assimilates allocated to the leaves during the vegetative stage compared to those from residue-free soil. During this stage net assimilation rate, relative growth rate, relative leaf area growth rate, and leaf area ratio became considerably greater than for controls. Although, relative leaf area growth rate was increased, which may have been due to more assimilates being allocated to the leaves, the greater magnitude of the increase in relative growth rate over that of the relative leaf area growth rate may account for the increase in the value of net assimilation rate. This would be possible if there was a reduction in respiratory losses, caused by the release of a respiratory inhibitor from the residues.

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LIST OF ABBREVIATION

cm	centimeter
DAE	days after emergence
dm	decimeter
g	gram
ID	inside diameter
L	leaf area of all leaves
LAR	leaf area ratio
LWR	leaf weight ratio
mg	milligram
mm	millimeter
NAR: E	net assimilation rate
NPK	nitrogen phosphorous potassium (fertilizer)
OD	outside diameter
PVC	polyvinyl chloride
RGR: R	relative growth rate
R_L	relative leaf area growth rate
SLA	specific leaf area
W	whole plant weight (dried)
W_L	dry leaf weight
W_P	dry pod weight
WP	wettable powder
W_R	dry root weight
W_S	dry stem weight

INTRODUCTION

Multiple cropping, as practiced by traditional small farmers in the tropics, allows crop intensification through maximum utilization of space and time (IRRI, 1973; Sanchez, 1976). The inherent problems in this practice have determined the evolution of the observed cropping patterns among small farmers in the tropical regions of the world.

Field experiments undertaken by the Multiple Cropping Department of the International Rice Research Institute (IRRI) at Los Banos, Philippines have shown the harmful effects of certain legumes (IRRI, 1973; Sikurajapathy, 1974) and dryland rice (Ventura and Watanabe, 1978) on the succeeding crop. The greatest effect was demonstrated to be on subsequent planting of the same crop; that is, in mungbean-mungbean, cowpea-cowpea or rice-rice (dryland) sequences. Sweet potato has been shown to be adversely affected by previous mungbean or cowpea crops and cowpea has been shown to be adversely affected by a previous mungbean crop.

The tolerance of mungbean to drought makes it a desirable crop in a rice-based cropping pattern where the farmer is dependent on available rainfall. The short growth duration of some of the newly developed varieties of mungbean allows two successive crops after rice, where

the available soil moisture is not sufficient for a second crop of rice. An understanding of the mechanism of the residual effect of mungbean on a subsequent mungbean crop will thus be of great benefit to small farmers since only with this understanding will it be possible to develop ways to eliminate or at least circumvent the problem.

Evaluation of the earlier experiments on residue effects conducted at IRRI and at the University of the Philippines tended to provide evidence supporting an allelopathic mechanism (Runeckles, 1974) although the role of nematodes and soil fungi was not conclusively ruled out (Runeckles, 1975). However, the experiments of Ventura and Watanabe (1978) showed that in mungbeans the inhibitory effects appeared to be directly dependent on microorganisms in the soil, although it was not determined whether the microorganisms caused direct damage to the root system as soil-borne pathogens or caused the production of toxic substances inhibitory to mungbean growth.

The phenomenon of the harmful effects of some crop residues is well-known to farmers. Farmers involved in cooperative experiments with IRRI in the study of rice-based cropping patterns are reluctant to include mungbean as a rotation crop (IRRI, 1975) especially when the field is intended for a tomato crop later in the season.

In the experimental fields at IRRI where this phenomenon has also been observed, the leaves and roots are left on and in the soil at harvest; the stems and pods are removed. The leaves are naturally shed as the pods mature, and accumulate and decompose on the surface of the soil. It is probable then that only roots and leaves are associated with the residue problem. However, the possibility also exists that materials are released into the soil during the active growth of the crop and that they persist long enough during the growth of the subsequent crop to induce harmful effects. Hence, the following possible mechanisms needed to be investigated:

1) the role of exudates from actively growing roots,
2) the role of decomposing root residues,
3) the role of decomposing leaf residues,
4) the interactive roles of both root and leaf residues.

With these possible sources of phytotoxin, it was deemed essential to study first the effects of a mungbean crop on the growth parameters of a succeeding mungbean crop grown under various conditions of potential transfer and source of phytotoxicants, rather than to attempt to identify the chemical compounds responsible. Hence a series of experiments was undertaken in order to establish that the phenomenon observed in the field could be reproduced under experimental greenhouse conditions, and subsequently to determine the relative roles of root

or leaf residues in inducing the effect.

Since previous field studies at IRRI had been essentially confined to the visual observations of symptoms of impaired growth and measurement of yield, it was anticipated that the analysis of growth throughout the growing period might reveal how the dynamics of growth were affected, whether or not severe symptoms or yield effects were observed.

An additional factor which required investigation related to whether the effect involved the transport of soluble phytotoxicants (e.g. see Rice, 1974) or was dependent upon physical contact between residues and crop roots as has been found to be the case in some systems (e.g. see Patrick et al., 1963). To achieve these various objectives, the following three experimental approaches were used:

1) The hypothesis that the growing mungbean plant produces phytotoxic root exudates was studied by transfering the leachates from the roots of plants in donor pots into receptor pots.

2) The hypothesis that only the decomposing materials, i.e., roots and leaves, produce toxic compounds that are water-soluble and transferable was studied by transfering the leachates from decomposing roots and leaves from donor to receptor pots.

3) The hypothesis that the residues have to be in contact with the roots of a succeeding crop was studied by

incorporating the dried and ground roots and/or leaves directly into the pots of the second crop.

LITERATURE REVIEW

Allelopathy

The term allelopathy refers to the detrimental direct or indirect biochemical effects of one plant (the donor) on the germination, growth, or development of another (receptor) plant (Putnam and Duke, 1978; Rice, 1974). The phenomenon, it should be pointed out, is not limited to the interactions between species. While the literature is replete with cases of biochemical interactions between species, several plants are also reported to exhibit autotoxicity as in the case for example of hedge bindweed *(Convolvulus sepium)* (Quinn, 1974), some varieties of rice (Sadhu and Das, 1971; Ventura and Watanabe, 1978), and alfalfa and timothy (Nielsen et al., 1960) to mention a few.

The influence of allelopathy in agriculture was recognized as early as the 5th century BC (Putnam and Duke, 1978). But it is only the last twenty years that its significance has caught the increasing attention of plant scientists and has led to an increasing number of publications and reviews, and to the treatise by Rice (1974). Allelopathy has been recognized in diverse plant habitats, for example, in deciduous forest (Lodhi, 1976), in old field succession (Rice, 1972; Wilson and Rice, 1968) and in the arid California chapparal (Muller et al., 1968). Because of the immensity of the subject of allelopathy in general, I limit the emphasis of this review to the problems that arise from crop residues.

In agriculture, there are numerous reports of the effects of crop residues. For example, Nielsen et al. (1960) reported the effects of alfalfa, corn, oats, potatoes and timothy on the germination and growth of six plant species. Patrick and Koch (1958) studied the effects of timothy, corn, rye, and tobacco residues in soil on the respiration of tobacco seedlings. McCalla and Army (1961) reported extensively on the effect of stubble-mulch farming.

In the study by Patrick and Koch (1958), it was found that the substances capable of inhibiting the germination, growth and respiration of tobacco seedlings arise under some conditions of decomposition. Species and stage of maturity of plant material, water content and pH of the soil, and length of decomposition period were among the important factors which influenced the production of phytotoxic products. Aqueous extracts of macerated undecomposed plant materials were not found to be toxic in their study.

However, the germination and growth experiments of Nielsen et al. (1960), using the standard germination technique in sand, indicated that aqueous extracts of crop residues of alfalfa, timothy, oats, corn and potato contained substances toxic to at least one of these crops and soybean. Compared to plants watered with deionized water, extracts from alfalfa residue caused the greatest reduction in shoot and root length and in percentage germination. They also

caused the greatest delay in germination. Timothy extract was not as harmful as that from alfalfa. Oats, corn and potato extracts were still less harmful, with potato extract the least. The crop species in this experiment also showed marked differences in their tolerance to the phytotoxic effects of the extracts. The order of decreasing resistance to the phytotoxic effects in general was as follows: alfalfa, corn, soybeans, peas, oats and timothy. However, alfalfa, timothy, corn and oats residues were shown to cause deleterious autotoxic effects. As indicated by the ratios of observations made on plants grown with and without extracts, timothy showed the greatest autotoxicity in terms of rate and percentage of germination, root and shoot length. Alfalfa extract caused the greatest effect on root growth but its effect on shoot growth and percent germination was less than that of timothy. Corn and oats showed much less autotoxicity.

The examples of the effects of crop residues raises the question about the effects of stubble-mulch farming. Extensive studies by McCalla and associates (McCalla and Army, 1961) have found that while the practice has been demonstrated to be of practical value in reducing soil erosion by wind and water, plant residues contain substances,and microorganisms in the decomposing stubble produce substances that may affect germination and growth. Guenzy and McCalla (1966) identified several phenolic compounds from stubble-mulch fields, and other workers

have also reported a number of phenolic acids and other related compounds which were shown to be phytotoxic to various crops from soils containing decomposing plant residues (e.g., Borner, 1960; Toussoun et al., 1968; Wang et al., 1967; Whitehead, 1963, 1964; Chou and Patrick, 1976; Chou and Lin, 1976).

The role of microorganisms in the residue problem is illustrated by the work of Cochran et al. (1977) with the crop residues common in eastern Washington. The study was undertaken to investigate the problem of reduced stand and plant vigor in the no-tillage or reduced tillage system of wheat production in the area. Mats (5-8 cmm thick) of crop residue of lentils, pea, wheat, barley and bluegrass were spread over bare field of Palouse silt loam soil. Water extracts of residues and soil beneath them were bioassayed weekly for wheat-seedling phytotoxicity and the residues were plated biweekly to determine the numbers of fungi, bacteria, and pseudomonads for the succeeding nine months, starting in September, 1975. All residues were found to produce wheat-seedling root inhibitors, but only after conditions became favorable for microbial growth. The residues were tested for the presence of patulin by thin layer chromatography but were found to be negative. No further attempt was made to identify the phytotoxin in this experiment. Patulin is a substance produced by *Penicillium urticae* Bainer found

to be common in stubble-mulch tillage in Nebraska (Norstadt and McCalla, 1968) and has been suggested to be the cause of as much as 50% inhibition of shoot growth in wheat.

Generally, the allelopathic compounds isolated from plant materials and from soil belong to the group known as secondary plant compounds. These include simple phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, cyanogenic glycosides and glusosinolates (Rice, 1974; Harborne, 1972). Rice (1974) proposed that the probable biosynthetic pathway of synthesis of the different classes of allelopathic chemicals arise through the acetate or shikimic acid pathways.

Allelopathic compounds are not unique chemically. These compounds have also been reported to be involved in several protective or defensive functions for the plant (Swain, 1977; Rice, 1974). For example, polysaccharides acylated with ferulic acid are suspected to be involved in the defensive functions of the plant cell wall against invading microorganisms (Wood and Granite, 1976) and as phytoalexins (Deveral, 1972; Swain, 1977), while ferulic acid has been identified as one of the allelopathic agents of decaying litter of dominant trees in a lowland forest community (Lodhi, 1978), in corn and rye residue decomposition (Chou and Patrick, 1976) and in the phytotoxic effects of decomposing rice residues in soil (Chou and Lin, 1976).

According to Putnam and Duke (1978) no one has proven that allelopathic chemicals are specifically synthesized as a result of an external stimulus. Whether the chemicals involved are end products of metabolism or are actually synthesized by the plant for a specific function is a major unanswered question in plant biochemical interactions. It is known that allelopathic chemicals are potentially autotoxic and are shunted into vacuoles to prevent autotoxicity (Whittaker, 1970).

Allelopathic effects are brought about in several ways (Rice, 1974; Putnam and Duke, 1978): by exudation of volatile compounds from living plant parts; by leaching of water-soluble toxins from above-ground parts through action of rain, fog or dew; by exudation of water-soluble toxins from below-ground parts; or by release of toxins through leaching from litter or as microbial-by-products resulting from litter decomposition. Phytotoxins once released must accumulate in sufficient quantity to affect other plants, must persist for some period of time, or must be constantly released in order to have lasting effect (Rice, 1974).

Diverse techniques have been employed to identify allelopathic chemicals which cause inhibitory effects on seed germination, plant growth and development. Rice (1974) has included detailed descriptions of many of the methods employed in the cases of allelopathy discussed in his treatise. Putnam and Duke (1978) have recently presented a comprehensive summary of the

methodology of allelopathy studies in their review of allelopathy in agroecosystems.

The most common method is that of cold-water extraction through simple soaking, for varying lengths of time, of either dried or live plant parts. The extracts are then usually filtered or centrifuged before bioassaying in petri dish or in flats of soil or sand or in nutrient solution. There are numerous reports of the effects of extracted compounds on germination, growth of roots or shoots, and other symptoms (Putnam and Duke, 1978). A variation of the cold-water extraction method is that of macerated plant parts placed in dishes containing moistened cellulose sponge that supports a filter paper on which indicator seeds are sown. Thus cold-water extraction and bioassay are carried out simultaneously. Many authors imply that cold-water extraction simulates the natural release of compounds by the action of rain on fallen plant material. However, Anderson and Loucks (1966) have demonstrated that extracts containing unknown and possibly high osmotic concentrations of non toxic compounds, such as sucrose, result in depressions of germination and early seedling development. In addition, at pH values between 5 and 6, sucrose solution (25 milliosmolar) has been demonstrated to reduce radicle growth of lettuce by as much as 75% (Chou and Young, 1974). Most of the studies in

allelopathy reported in the literature do not take into consideration the roles of osmotic concentration and pH of extracts used in the bioassays.

Bioassaying by means of petri dish or nutrient solution techniques of materials extracted by boiling water (Jackson and Willemsen, 1976), autoclaving (Kommedahl and Ohman, 1960), or organic solvents (Friedman and Horowitz, 1971) are other methods which have been used. Hot water and autoclaving eliminate microbial decay while allowing increased diffusion of soluble compounds into the acqueous phase. The use of organic solvents in the extraction process permits a larger number of compounds to be isolated which may be phytotoxic. But, in all these methods of extraction, the phytotoxic materials isolated may include substances that are not necessarily the cause of problems under natural conditions (Putnam and Duke, 1978).

In detecting the presence of inhibitory substances from below-ground parts, various techniques have been employed. Each method used attempts to prevent water and nutrients from being a limiting factor in the growth of test plants so that the observed effects can clearly be attributable to chemical toxicity. The most common method is exemplified by the stairstep system used by Bell and Koeppe (1972) in studying the non-competitive effects of giant foxtail (*Setaria faberii* Herm.) on the growth of corn. The system involves the growing of donor and recipient plants in sand in pots arranged alternately in

stairsteps so that the nutrient solution flows from donor to recipient and back to a reservoir. The flow of nutrient solution is set to a number of cycles per day. Since the physical aspects of competition are eliminated in this system, possible allelopathic effects resulting from the exudation and leaching of phytotoxins from one species can be studied directly.

Some of the other methods used in detecting inhibitory substances from root exudates are: (a) growing donor plants for specific times, leaching the sand, and evaluating the leachates on recipient plants in petri dishes, or in soil or sand (Fay and Duke, 1977), (b) growing both donor and recipient plants in sand and evaluating the effects before competition for other growth factors occurs (Putnam and Duke, 1974).

A straightforward method has been used in examining the release of organic inhibitory substances from decomposing plant materials. Either dried or live plant materials are placed in or on soil for selected time periods before bioassaying with recipient plants (Wilson and Rice, 1968; Rice, 1972). Although certain studies have identified specific fungi involved in the decomposition of plant materials and have evaluated by-products of fungal metabolism on plant growth (Norstadt and McCalla, 1962; 1968) there is difficulty in determining whether the toxic effect comes from the plant, the microorganism, or is the result of an additive or synergistic effect of both (Rice, 1974).

The methodology for the study of allelopathy has thus been varied. There is still a need to develop specific and reliable procedures for the isolation of suspected compounds, and bioassay techniques that are effective and prove the existence of toxic components more definitively than the extraction methods used to simulate effects observed in nature.

Growth Analysis

Numerous excellent reviews have been written on this subject and discussion can be found in Evans (1972), Radford (1967), Richards (1969) and Sestak et al. (1971).

Growth analysis can be described by a set of equations (Evans, 1972) representing the performance of the plant's functional parts; thus:

RGR	$\doteq$	NAR	x	LAR
$(dW/dt)(1/W)$	=	$(dW/dt)(1/L)$	x	L/W

LAR can be further divided into its components:

$$LAR = SLA \times LWR$$
$$= L/W_L \times W_L/W$$

where RGR = Relative Growth Rate; R

NAR = Net Assimilation Rate (also called ULR, Unit Leaf Rate); E

LAR = Leaf Area Ratio

W = Whole plant dry weight

L = Leaf area of all leaves

SLA = Specific Leaf Area

LWR = Leaf Weight Ratio

W_L = Leaf dry weight

Relative growth rate is defined as the rate of increase in dry matter content of the plant with respect to the amount of dry matter already present. Its use permits the comparison of the growth of plants of different sizes, since RGR is an overall measure of plant performance.

The relative growth of assimilatory apparatus (and other plant parts) is defined similarly to the relative growth rate of dry matter accumulation (Sestak et al., 1971). Whitehead and Myerscough (1962) reported that α, the ratio of relative growth rate (dry matter basis) to relative rate of leaf area increase ($\alpha = R/R_L$, where R_L is the relative leaf area growth rate), is a parameter of considerable importance. The value of α indicates the amount of dry weight increment that is in excess of the amount required to maintain the morphogenetic proportions of the plant as an efficient photosynthetic unit. When $\alpha = 1$ all of the dry weight accumulated is used up in maintaining the overall morphological form of the plant. The later stages of morphogenesis which entail the production of storage organs, and reproductive structures thus usually require a surplus of assimilates, i.e. $W(\alpha - 1)$ in order to produce flowers, fruits, etc. from an assimilatory apparatus of finite size.

Growth analysis has classically been done by computation from averages of discrete harvests taken at several intervals of time during the growth duration of the plant. As a consequence, resulting calculations have been inaccurate and imprecise. A curve-fitting approach using stepwise multiple regressions (Radford, 1967) has been shown to be more accurate. But this was very laborious and time-consuming prior to the availability of computing facilities and computer programs such as those of Hunt and Parsons (1974; 1977).

The technique of growth analysis, since its inception more than 50 years ago, has been used to study growth characteristics and to quantify the accumulation of dry matter of field crops grown under various conditions.

Koller, Nyquist and Chorush (1970), in studying components of dry matter accumulation in field soybeans, found that RGR of individual plant fractions (leaf, stem, etc.) steadily decreased as the plant matured. At any given time the most recently initiated plant fraction had the greatest RGR. Total above-ground RGR declined until early pod formation when it peaked concurrently with an increase in NAR. They interpreted the increase in NAR as a response of the photosynthetic apparatus to an increased demand for assimilates due to the rapid growth of the seed fraction.

Buttery (1969) studied the effects of population density and fertilizer application on field-grown soybeans, and found that, regardless of treatment, there was a decline in NAR and RGR towards maturity. The decline was attributed primarily to increasing leafiness.

Thorne (1960) reported that, under controlled environment, NAR of sugar-beet, potato, and barley fell approximately linearly with time. During 5 weeks, NAR of sugar-beet and potato decreased by 20 and 50 percent respectively. NAR of barley remained approximately constant for 4 weeks but was halved during the subsequent weeks. RGR, R_L and LAR fell with time at similar rates for all three crop species.

Some stress factors have been shown to have contrasting influences on growth parameters. For example, Last (1962) reported that the changes in root development and leaf area caused by mildew(*Erysiphe graminis* D.C.) on barley were associated with similar decreases in NAR. From 12 to 68 days after inoculation the mean NAR was 226.6 mg/dm^2/w in mildew-free controls and 166.0 mg/dm^2/w in the inoculated series. In young tomato plants subjected to wilting treatments of short duration, Gates (1955) reported that NAR and RGR was reduced during the period of wilting but the growth parameters were greater than for control plants upon rewatering. During wilting, higher stem weight ratios and lower leaf weight ratios developed than in the control, whereas after wilting, leaf weight ratios were higher than stem weight ratios. However, there was no indication that the recovery effect was complete at the final harvest. Gates (1955) interpreted these treatment effects as a tendency towards senecence during wilting and a return to a more juvenile condition upon rewatering. He concluded that the changes in weight ratios were due to modifications of the normal pattern of translocation between plant parts.

Tsiung (1978), in a study on the response of mung-bean to sowing dates in the Malaysian Borneo state of Sarawak (4^{o}07' N; 113^{o}57'E), applied the technique of growth analysis to characterize the marked growth differences among the sowing dates. A Philippine variety, CES-55 used

in this study, was sown at the beginning of March, May, July, August and September, 1976. Although there were marked differences in plant growth, the phenology was similar for all planting dates: flowering at 33 days after sowing, ripening of bean pods at 51 days and maturity by day 70. Dry matter accumulation was also similar--- slow during the first 20 days, followed by a rapid increase once flowering and pod-setting commenced, attaining a maximum at day 60.

Tsiung (1978) found out that the pattern of changes in RGR in all sowing dates was characterized by an increase from day 15 to a peak at day 25 followed by a rapid, smooth decrease thereafter, attaining a negative value at day 65. NAR behave similarly except that from day 25 to 55 the decrease was at a slower rate but dropped to a negative value also at day 65. The decrease in RGR was attributed to a faster rate of decline in LAR (56%) than in NAR (36%).

As far as I can determine, there is no report on the application of growth analysis on plants grown under stress of allelopathy.

MATERIALS AND METHODS

The experiments were conducted in the greenhouse of the Department of Plant Science, University of British Columbia, at a latitude of 49°16'N. The temperature regime was established such that minima ranged between 20° and 22°C and maxima between 28° and 30°C. During winter months supplementary lighting was provided for 12 hours per day by means of five 400-watt high pressure Sodium-vapor (Lucalox) lamps arranged in a row 1.6 m above the center of the four-row pot arrangement. The rows of the pots were spaced such that all the pots were located within the 1.2m-wide area where the light intensity was uniform. The illumination measured 140 cm below the lights at 16:00 on a overcast day was 518 $\pm$ 13 and 534 $\pm$ 17 foot-candles on the north and south side rows respectively. The intensities over the two middle rows were 620 $\pm$ 15 and 610 $\pm$ 15 foot-candles. Outside the greenhouse at the time of the above measurements the intensity was 156 foot-candles. The relative humidity fluctuated between an average maximum of 80% and an average minimum of 55%.

General Management

All the plants were grown in 4800 cm^3 growing medium. The river sand used in Expts. 1 and 2 was washed thoroughly with water until the washings were clear. The

soil used in all experiments was taken from the steam-sterilized potting soil supply in the greenhouse. Gravels and other matters larger than 1 cm diameter were screened out. Added amounts of fertilizers were shovel-mixed in measured batches corresponding to one replication.

In Experiments 1 and 2, the mungbean cultivar used was MG50-10a (green-seeded). Since an earlier planting of this cultivar had shown wide variability (some were purple-based and tend to be viny), heavy seeding rates were used and off-types were rogued out 10 days after emergence. Yellow-seeded MG50-10a was used in Experiments 3a and 3b. Again, two types were observed: dull yellow and glossy yellow. The latter were selected and were observed to be uniform.

Maintenance of all experiments consisted of daily inspection for moisture status of the pots, temperature and humidity extremes, and occurence of pests. The soil was not allowed to dry up on the surface nor was it allowed to become water-logged. A hygrograph and thermograph placed near the center of the greenhouse monitored the daily humidity and temperature ranges. The desired temperature range was maintained by means of the automatic temperature controls of the greenhouse. Humidity was kept above 50% by periodic sprinkling of water around the experimental area. The plants were sprayed with propargite 30WP at 1.25 g/liter, a miticide, as mite infestations were observed after pod set. Regular fumigations of the greenhouse complex were sufficient to control other

pests, particularly the greenhouse whitefly.

Experiment 1

The objective of this experiment was to determine whether materials leached from the roots of mungbean plants growing in sand culture during their growing period and transferred continuously to pots of steam-sterilized soil would accumulate and influence the growth of mungbean plants sown subsequently in the recipient soil.

The experimental set-up was composed of: a) a nutrient solution container with distribution tubing; b) donor pots with sand as the rooting medium; c) recipient pots with fertilized, steam-sterilized potting soil as the rooting medium. The pots and nutrient supply were arranged as a stairstep system in which each donor pot leached into a recipient pot by means of tubing.

The general arrangement is shown in Fig. 1a and in close-up in Fig. 1 b. The distribution and regulation of the nutrient flow to the individual pots were accomplished in the following manner: PVC black tubing (1.27 cm ID) of appropriate length was connected at one end via a check valve to the nutrient solution container; the other end was connected via a plastic pipe reducer to a flexible tygon tube (0.64 cm OD) open at the other end. This tygon tube was long enough to serve as a "standpipe",

Figure 1. General arrangement and close-up view of the root leachate transfer experiment.

to balance the pressure head of the nutrient solution in the container and to avoid the problem of air trapped within the PVC tubing. Along the length of the PVC tube, fine PVC leader tubes (1.2mm ID) were connected, via brass insert couplers, to deliver the nutrient solution drop by drop to the donor pots. The outlet ends of the leader tubes were fixed at a height to deliver 6 drops per minute to each donor pot. Two leader tubes were provided per pot to distribute the moisture more evenly on the surface of the rooting medium.

Glazed ceramic pots (17 cm dia) with a drain hole (2.54 cm dia) on the side near the bottom were used for both donor and recipient pots. A semi-flexible polypropylene tube (0.64 cm OD) of appropriate length was fitted tightly at one end into a hole drilled through a rubber stopper inserted into the hole in the donor pot. The other end of this tube was plugged. Two leader tubes were connected via brass insert couplers near the plugged end, to deliver leachate to each recipient pot, as shown in Fig. 1 b.

Nutrient solution. Nutrient solution No. 1 as described in California Agri. Expt. Sta. Cir. 347 (Hoagland and Arnon, 1950) was allowed to flow through each experimental unit. The flow from the nutrient supply was regulated such that the leachate solutions were delivered from the donor to the recipient pots at a rate which avoided excessive flow through the recipient pots to waste. The amount of solution that drained from the

recipient pot was minimal and was not recirculated back to the system.

Donor pots. It was expected that the low absorbing capacity of sand would allow the maximum transfer to the recipient pots of whatever exudate was released from the plants in the donor pots. However, at the conclusion of each period of leachate transfer (see section on Recipient pots, below) the donor pots were disconnected and flushed with two volumes of water to wash out remaining exudates. The washings were saved and used to water the recipient pots until consumed. The donor pots were seeded to a final stand of 4 plants/pot except for the controls.

Recipient pots. The use of steam-sterilized soil minimized the influence of microorganisms and excluded contamination by weed seeds which might have germinated and influence the result. Seeding to a final stand of 4 plants per pot was done 7 days after the end of the leaching period, which approximates the time interval between successive plantings in the field. Slow-release fertilizer (Osmocote, 14-14-14 NPK) was added at the rate of $74g/dm^3$ to insure an adequate supply of nutrients, since the recipient plants were watered from the tap after the stored leachates had been consumed. Two periods of leachate accumulation were investigated. In one, leachates were transferred up to the end of the vegetative stage of growth of the donor plants (about 28 days). In the other, leachate transfer continued to maturity (about 70 days). A separate set with no plants in the donor pots served as the control, for each leaching period.

Experimental design. Four treatments consisting of two periods of leachate accumulation and one untreated control for each period comprised a replicate. A treatment consisted of two pots: donor and recipient. There were five replications for a total of 40 pots in a randomized complete block design.

Experiment 2.

This experiment involved the leaching of decomposition products of roots and leaves. The same experimental system was used as in the first experiment, except that the donor pots contained decomposing residues which leached into recipient pots. The plants of the donor pots in Experiment 1 were grown to maturity after which the tops were clipped off. These served as the root residue donor pots. The leaves from two of the pots in a replicate were pooled and incorporated into one of the blank donor pots of the same replicate. These pots served as the leaf residue donor pots. The donor pots thus consisted of two pots with root residues, one pot with leaf residues and one continuing control pot, per block. All the plant residues in these pots were allowed to decompose for one week before leaching. Leaching was done for thirty days by watering the donor pots from the tap just enough to soak the recipient pots every other day. The control

donor pot was treated in the same way as the pots with residues.

The recipient pots in this experiment had the same soil source as in Experiment 1. The same rate of fertilizer was also added and seeding to a final stand of 4 plants/pot was done immediately after the 30-day leaching period. Again, a randomized complete block design was used.

Experiment 3a.

The objective of this experiment was to determine whether direct contact between the roots of a subsequent mungbean crop and the root and leaf residues from a previous mungbean crop grown in soil was a requirement in order for the second crop to be affected. It was conducted in two stages. This approach simulated the sequential cropping as practiced in the field.

Establishment of first crop. Pots of soil with fertilizer added at the same rate as in Experiment 1 were seeded to a final stand of 4 plants/pot. (Pots were thinned 5 days after sowing). The same number of pots of soil were also prepared along with the cropped soil and laid fallow for the duration of the first crop. The plants were grown to maturity, i.e., when most of the pods had turned black, after which the leaves were collected,

dried, ground, and incorporated back into soil of the corresponding pots, which contained the root residues *in situ*.

Second crop. The pots of cropped soil were divided to provide two "incubation" periods. Seeding to a final stand of 4 plants/pot was done after one-week and after three-week incubation periods. A corresponding number of fallow pots were also seeded for each incubation period to serve as controls. The growth of the plants was monitored by sampling at four stages:

1) 14 days after emergence (DAE), when the first trifoliate leaves had expanded;

2) 28 DAE, flowering stage;

3) 42 DAE, pod-filling stage;

4) 73 DAE, maturity -- when 70% of the pods had turned black.

Experimental design. The experiment consisted of 4 treatments, 4 sampling dates, and 5 replications, a total of 80 pots. Since the treatments were composed of two groups of plants with a two-week age difference, it was thought that a random arrangement of all the pots would have been disadvantageous to the younger plants because of possible shading by the older plants. A split-plot design with treatments as the main plot in strips and sampling dates as subplots was therefore used.

The vacant spaces created as sampling was done were filled by "filler pots" of plants seeded at the same time as the experimental plants. This was done in order

to minimize the edge-effect of the vacant spaces created by harvesting. Border pots of plants were also placed around the whole experimental area.

Experiment 3b.

In this experiment I studied the growth of a second mungbean crop, comparing soil which contained both root and leaf residues against soil which contained either root residues alone or leaf residues alone. In this way the leaf residue effect was separated from the effect of root residue.

The same general procedure was followed as in Experiment 3a, i.e., the establishment of the first crop followed by the second crop. The treatments were established by dividing the number of pots of cropped soil and fallow soil into halves. The leaf residues from one half of the cropped pots were incorporated into the corresponding number of pots of the fallow (control) soil. This made a total of 20 pots with roots plus leaves, 20 pots with roots alone, 20 pots with leaves alone, and 20 pots without residues. The second crop was seeded after 12 days' incubation of the plant residues. The pots were arranged in a randomized complete block design consisting of 4 treatments, 4 sampling dates and 5 replicates, a total of 80 pots.

Collection of data

For all harvests, the plants were cut off at the crown of the roots. Leaves (including petioles) were separated from the stems, and leaf area measurements were made immediately with a Hayashi Denko AAM-5 automatic photoelectric integrating area meter. Where root weight was recorded, roots were separated from soil by means of 5-mm screenwire mesh and subsequent washing in water with the aid of a fine kitchen sieve. Washed roots were blotted in paper towels before bagging. Leaves, roots, stems, and pods from each pot were bagged and labelled separately before oven drying at 70°C for at least 48 hours. The oven-dried samples were cooled to room temperature sealed in plastic bags prior to weighing. Each plant part (stem, leaves, etc.) was weighed separately and weights were expressed in g/pot of 4 plants. The leaf area reading in cm^2 was converted into dm^2 and was expressed as dm^2/pot of 4 plants.

For Experiments 3a and 3b, SLA, LWR, LAR values were calculated by means of a desk calculator and were tabulated together with leaf area (L), leaf weight(W_L), stem weight (W_S), pod weight (W_P), root weight (W_R) and total plant dry weight (W), which is the sum of all component weights. These values were subjected to analysis of variance through the facilities of the UBC Computing Centre. No statistical analysis were done on the data gathered from Experiments 1 and 2 since the familiar symptoms of the effect of

a previous crop were not observed and the plants in the receptor pots were uniform in size and appearance.

Calculations of the growth parameters R, R_L, E and α for Experiments 3a and 3b were first done with the traditional method described by Evans (1972) and Sestak et al. (1971). But since it was difficult to ascertain from the calculated values the actual pattern of growth because of lack of sampling points along the growth curve, the curve-fitting approach (mentioned on page 17) was resorted to. Cubic polynomial equations (see Appendix 1) were fitted to the changes in mean total dry weight (W) and leaf area (L) with sampling time (t). These can be represented (Sestak et al., 1971) as the following:

$$W = f_1(t) = a + bt + ct^2 + dt^3$$

$$L = f_2(t) = a' + b't + c't^2 + d't^3$$

from which R, R_L, E and α can be derived thus:

$$R = \frac{df_1(t)}{dt} \cdot \frac{1}{f_1(t)}$$

$$R_L = \frac{df_2(t)}{dt} \cdot \frac{1}{f_2(t)}$$

$$E = \frac{df_1(t)}{dt} \cdot \frac{1}{f_2(t)}$$

$$\alpha = R/R_L$$

In generating the polynomial equations, a small dummy number was used at t=0 in both Experiments 3a and 3b. Figures 13 and 14 show the fitted curves for W and L. The actual and fitted values for Experiment 3a are presented in Appendices 2 and 3. In Experiment 3b the fitted values were exactly the same as the actual values since there were only three sampling points. The means of the actual values for W, W_L, W_R, W_S, W_P, and L for Experiment 3a and 3b are presented in Appendices 4 and 5.

For convenience in presentation, the units of the following parameters were changed:

R	$g.g^{-1}.day^{-1}$	to	$mg.g^{-1}.day^{-1}$
E	$g.dm^{-2}.day^{-1}$	to	$mg.dm^{-2}.day^{-1}$
R_L	$dm^2.dm^{-2}.day^{-1}$	to	$cm^2.dm^{-2}.day^{-1}$

Calculation of α was based on the original units of R and R_L.

RESULTS

The current use of the term allelopathy refers to the harmful effects of higher plants of one species (the donor) on the germination, growth, or development of plants of another (receptor) species (Putnam and Duke, 1978). In the following discussions the use of the term is extended to apply to the detrimental effects of a previous crop (the donor) on the growth and development of the succeeding crop (receptor) of the same species.

Leachate transfer.

Experiment 1 was undertaken in an attempt to demonstrate whether water-soluble toxins are exuded from healthy intact roots of mungbean, accumulate in rhizosphere, and may be transferred and made to accumulate in a soil medium without loss of toxicity.

The receptor plants did not show any obvious, visible differences from the controls from the time of germination to maturity. The weight of tops and leaf areas of the receptor plants are presented in Tables 1 and 2 respectively. Inspection of these data reveals no significant effects of treatment *per se*, and no differences between the 28-day and 70-day leaching periods.

Table 1. Top weight (g/pot of 4 plants) of mungbeans grown in soil that had received leachates from mungbeans growing on sand culture. Harvested at maturity. (70 DAE)

Treatment	Replication					Mean
	1	2	3	4	5	
1. 28-day leaching	22.0	30.4	23.2	33.6	31.6	28.2
2. Control for Treat. No. 1	33.6	31.6	22.0	30.4	21.2	27.8
3. 70-day leaching	33.2	28.0	24.0	32.0	24.8	28.4
4. Control for Treat. No.3	31.6	23.4	22.1	30.3	33.4	28.2

Table 2. Leaf area (dm^2/pot of 4 plants) of mungbeans grown in soil that had received leachates from mungbeans growing on sand culture. Harvested at maturity. (70 DAE).

Treatment	Replication					Mean
	1	2	3	4	5	
1. 28-day leaching	20.9	27.9	21.9	30.4	27.1	25.6
2. Control for Treat. No. 1	29.4	27.8	19.8	26.4	18.8	24.4
3. 70-day leaching	28.9	24.9	18.7	28.2	20.2	24.2
4. Control for Treat. No. 3	28.3	20.9	18.9	27.6	30.2	25.2

Experiment 2 attempted to show if there are water-soluble phytotoxins that would leach out of decomposing residues after death.

The donor plants which were grown to maturity in Experiment 1 were used in this experiment. Their root or leaf residues were allowed to decompose in the original sand medium and leachates were transferred into soil in which the receptor plants were grown. Tables 3, 4 and 5 show top weights, leaf areas and heights respectively of the receptor plants harvested at the flowering stage, as affected by leachate accumulation from decomposing leaves and roots. As in Experiment 1, there were no visible differences between the treated plants and the controls, from the time of emergence to harvest date. Similarly, the data reveal no significant effects of treatment. However, it is of interest to note that the data suggest a greater effect of leaf rather than root residues.

Table 3. Top weight (g/pot of 4 plants) of mungbeans grown in soil that had received leachates of decomposing roots and leaves. Harvested at flowering stage. (35 DAE)

Treatment	Replication					Mean
	1	2	3	4	5	
1. Decomposing root	11.0	15.2	11.6	16.8	15.8	14.1
2. " leaves	16.8	15.8	11.0	15.2	10.6	13.9
3. Control	16.6	14.0	12.0	16.0	12.4	14.2

Table 4. Leaf area (dm^2/pot of 4 plants) of mungbeans grown in soil that had received leachates of decomposing roots and leaves. Harvested at flowering stage. (35 DAE).

Treatment	Replication					Mean
	1	2	3	4	5	
1. Decomposing root	18.6	16.4	17.1	18.2	18.8	17.8
2. " leaves	21.4	19.6	14.2	18.2	14.2	17.5
3. Control	19.6	16.2	13.9	19.8	20.4	18.0

Table 5. Average plant height (cm) of mungbeans grown in soil that had received leachates of decomposing roots and leaves. Harvested at flowering stage. (35 DAE).

Treatment	Replication					Mean
	1	2	3	4	5	
1. Decomposing root	38.4	38.1	38.7	36.9	37.8	38.0
2. " leaves	41.0	41.3	34.3	37.8	32.4	37.4
3. Control	42.0	37.8	36.3	40.6	37.5	38.8

Sequential cropping.

Since none of the leachate-transfer experiments showed the typical symptoms of the effect of the donor on the receptor plants, it was deduced that the problem does not involve a mere straightforward release of phytotoxins which can be easily leached from the growing medium. With this in view, I attempted to simulate as closely as possible the conditions as practiced in field cropping. Where the problem is observed, the second crop is established in quick succession to the first, with the leaves and root residues of the first crop worked into the seedbed.

Experiment 3a was primarily designed to compare the growth of mungbeans in fallow soil and cropped soil into which were incorporated root and leaf residues of a previous mungbean crop. A second objective was to find out if the length of incubation of the residue had an influence on the problem.

Germination in both cropped and fallow soil was uniform and occured essentially at the same time. But the treatments showed observable differences immediately after emergence. Those plants grown in cropped soil showed the typical symptoms observed in the field. Thus, expansion of the cotyledonary leaves was inhibited (Figs. 2 and 3), growth was stunted (Figs. 4 and 5) and root development was severely reduced (Figs. 6 and 7) in all the plants

Figure 2. Reduced size of mungbean seedlings at 7 DAE grown in cropped soil with root and leaf residue incorporated and incubated for one week. Note the early development of the first trifoliate leaves in the no-residue soil.

Figure 3. Reduced cotyledonary leaf expansion of mungbean grown in the residue treated soil. 14 DAE.

Figure 4. Stunted growth of mungbean grown in cropped soil with root and leaf residue incorporated and incubated for one week. 14 DAE.

Figure 5. The same treatment as in Figure 4 at 28 DAE. Grid lines in the above photographs are 15.24 cm apart.

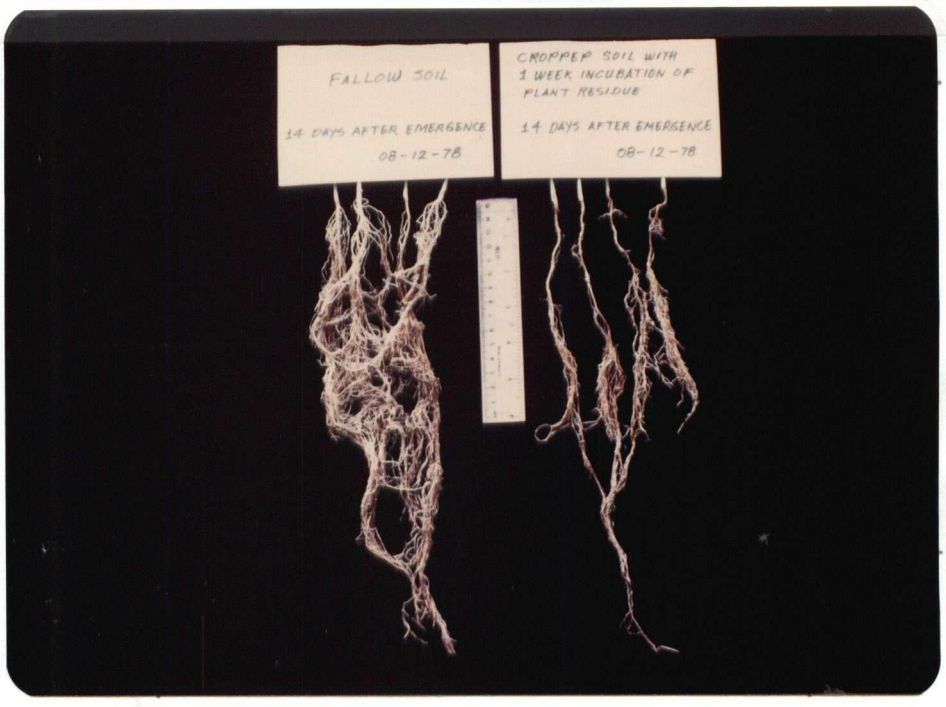


Figure 6. Root development of mungbean shown in Figure 4.

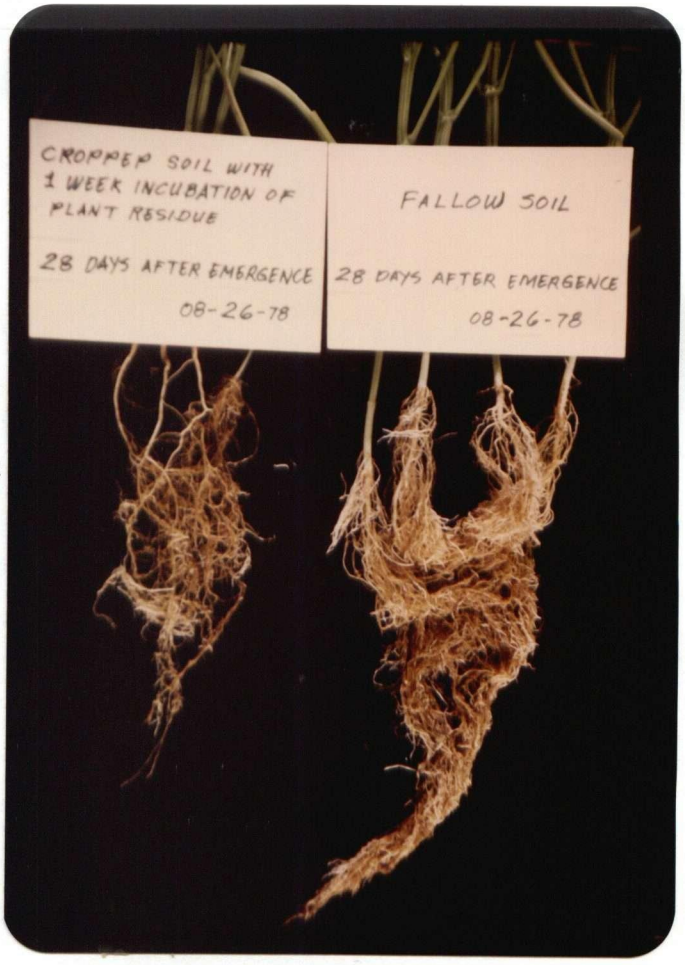


Figure 7. Root development of mungbean at 28 DAE of the same treatment as in Figure 4.

of the cropped soil. Examination of the seedlings from cropped soil about 3 days after emergence showed that the base of the stem was thickened and curled and the tap root did not develop (Figs. 8 and 9) as compared to normal seedlings of the same age (Fig. 10).

Total dry weights, component weights (leaves, stems, roots, and pods), leaf areas, LAR's, LWR's and SLR's were subjected to analysis of variance. Comparisons and contrasts of treatment effects were done in the following manner:

a) one- vs 3-week incubation

$$\bar{x}_1 = 1/2(T_1 + T_2) \text{ vs } \bar{x}_2 = 1/2(T_3 + T_4);$$

b) with vs without residue

$$\bar{x}_3 = 1/2(T_1 + T_3) \text{ vs } \bar{x}_4 = 1/2(T_2 + T_4);$$

where T_1 = Treatment with 1-week incubation of residues;
T_2 = Control for T_1;
T_3 = Treatment with 3-week incubation of residues;
T_4 = Control for T_3.

Table 6 summarizes the analyses of variance done on the component and total dry weights, and contrasts the effects of the treatments (with vs without residues) and the effects of the treatments (one- vs three-week incubation period). Obviously, the root and leaf residues severely reduce the component weights and hence the total dry weights of the receptor plants. The extent of the reductions caused by the incorporation of the residues is presented

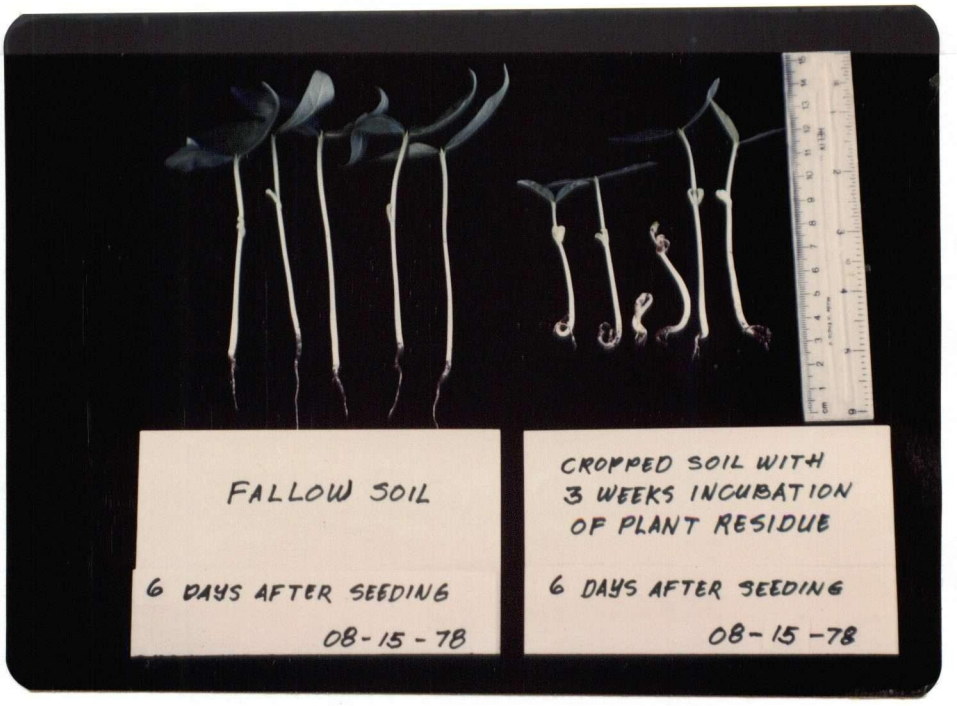


Figure 8. The effect of root and leaf residues, incorporated and incubated for three weeks into the soil, on the development of mungbean seedlings. 3 DAE (6 days after seeding). Note the slightly thickened and curled basal portion of the hypocotyl in contrast to the untreated soil (fallow).

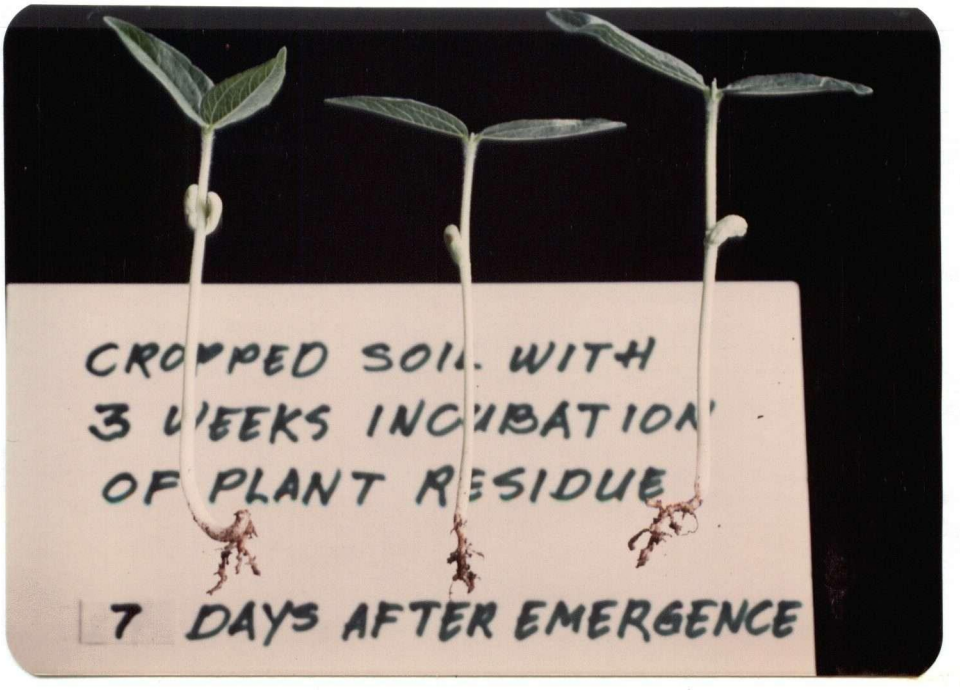


Figure 9. The same treatment as in Figure 8 at 7 DAE. Note the abnormal development of the primary root. Above magnification is twice that of Fig. 8.

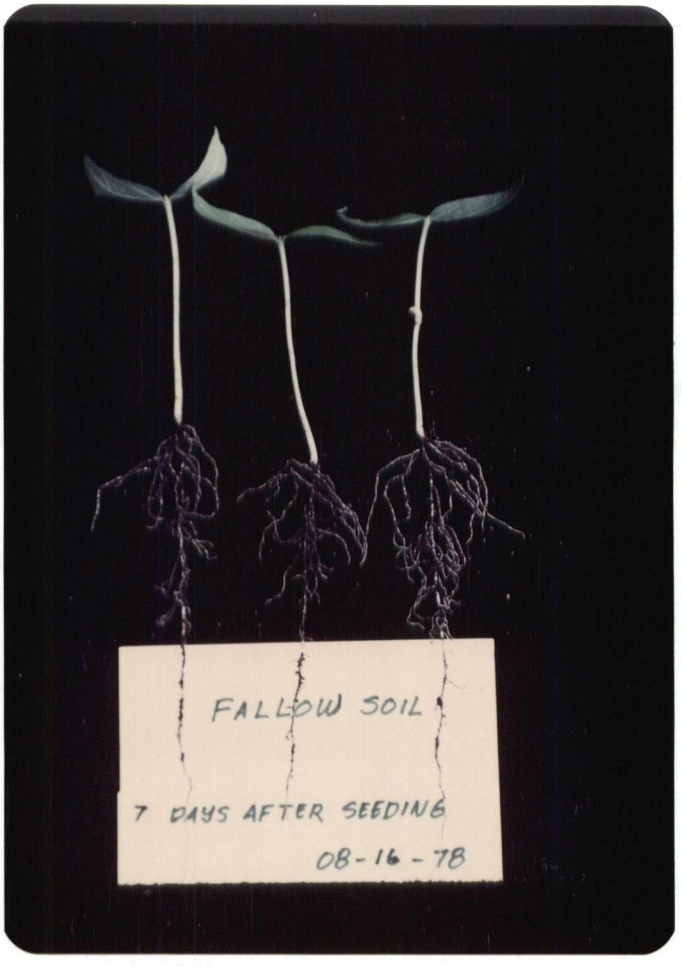


Figure 10. Normal seedling development of mungbean at 7 DAE. Magnification is 1:4.

in Figure 11. The linear regression lines are highly significantly different (Table 6). The primary data are presented in Appendix 4.

Table 6. Summary of contrasts between effects of the treatments: with vs without residue, and the effects of the treatments: one- vs three-week incubation period.

Variables	with vs without residue	one- vs three-week incubation	incubation x residue interaction
Total dry weight	**	**	ns
leaf area	**	**	ns
leaf weight	**	**	ns
stem weight	**	ns	*
root weight	**	**	ns
pod weight	**	*	ns
LAR	*	ns	ns
LWR	*	ns	ns
SLA	ns	ns	ns

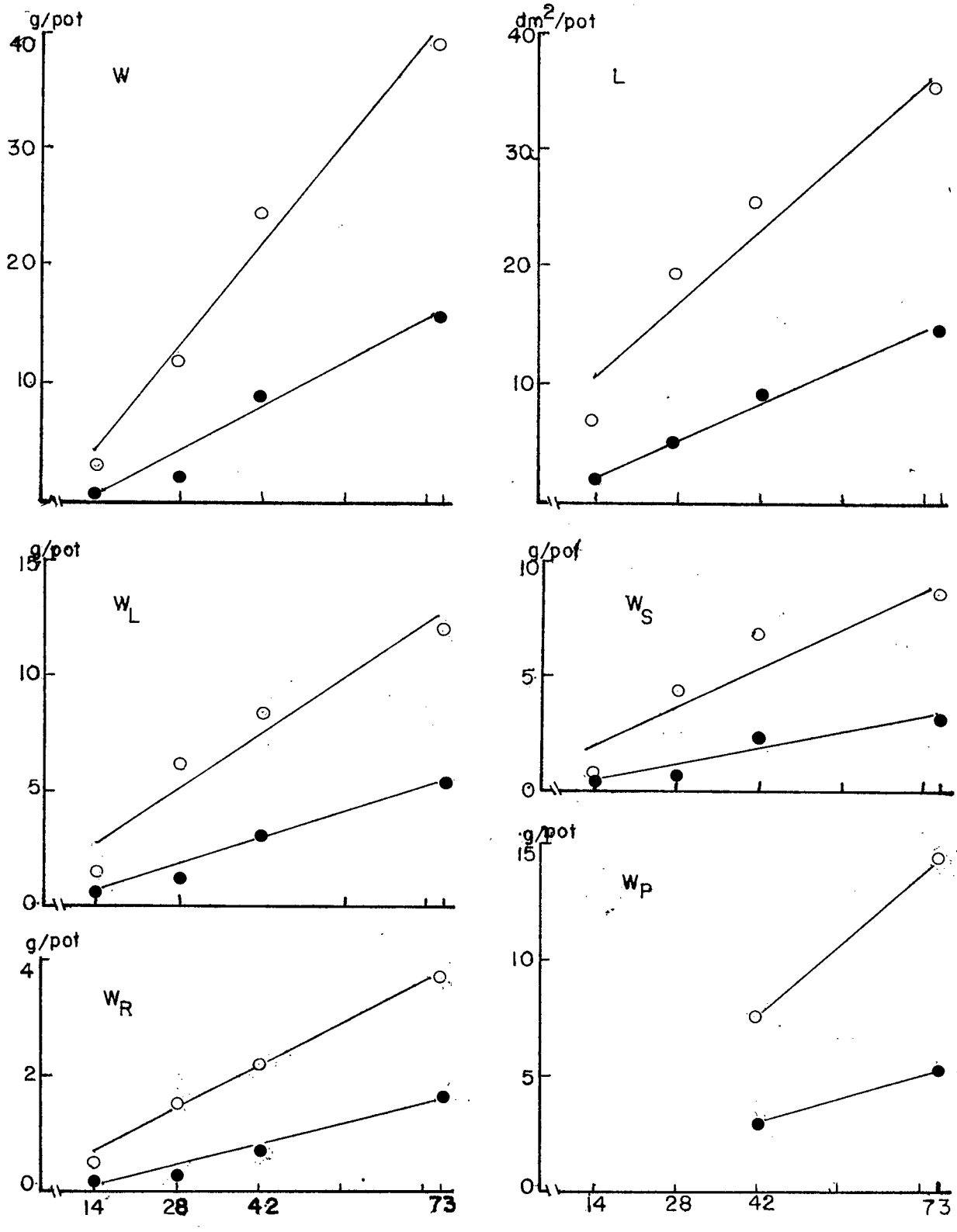
** Statistically significant at 1% level.

* Statistically significant at 5% level.

ns Non-significant

Incubation of the residues for up to three weeks appears to enhance its deleterious effects on the

Figure 11. Growth of mungbean in cropped soil with leaf and root residues of the previous crop incorporated (●), and in soil that was laid fallow, without residues (o). Total dry weights (W), leaf (W_L), stem (W_S), root (W_R), and pod (W_P) weights are expressed in g/pot of 4 plants. Leaf area (L) is expressed in dm^2/pot of 4 plants. Each point represents the mean across incubation periods. For each parameter, the slopes of the linear regression lines are highly significantly different.


g/pot
W
40
30
20
10
dm²/pot
L
40
30
20
10
g/pot
W_L
15
10
5
0
g/pot
W_S
10
5
0
g/pot
W_R
4
2
0
g/pot
W_P
15
10
5
0
14
28
42
73
DAYS AFTER EMERGENCE

accumulation of dry matter in all parts of the plant and on leaf area (Table 7). The only exception is stem weight (Table 6). This lack of effect of incubation period on stem weight probably accounts for the sole significant interaction between incubation period and residue (Table 6).

Table 7. Comparison of means across all harvests for 1-week and 3-week incubation periods with leaf and root residues.

Variables	Incubation period			
	one week		three weeks	
	Treated	Control	Treated	Control
	(g/pot of 4 plants)			
Total dry weight	9.20	20.80	5.03	18.76
leaf weight	3.54	7.53	1.66	6.69
stem weight	2.17	5.08	1.07	5.35
root weight	0.93	2.12	0.46	1.89
pod weight	2.57	6.08	1.84	4.84
	(dm^2/pot of 4 plants)			
leaf area	10.51	20.80	5.08	20.45

Since the analysis of variance (Table 6) revealed no major interactions between the two treatment sets, the mean values of the derived growth parameters for the one- and three-week incubations were initially compared

to the means of the corresponding controls (Fig. 12). The effects of residues are clearly shown, with the major differences occuring during the early stages of growth. The exception is the effect on relative leaf area growth rate, in which there is a consistent stimulation caused by the presence of residues throughout the growing period. However, it should be pointed out that, while the rate of leaf area expansion may have been stimulated, the total leaf areas of the plants grown in the presence of residues were consistently and substantially less than those of the controls, as revealed by Fig. 13 and 14.

The early peaks in the fitted curves for R, E, LAR, and α for residue-grown plants (Fig. 12) are therefore the result of the delay in the onset of appreciable growth. Thus during the first 14 days of growth, these plants accumulated little dry matter, so that in relative terms, their slow growth during the subsequent 14 days nevertheless revealed itself through relative growth rates considerably greater than those of the controls.

Figures 13 and 14 present the actual and fitted data for W, L and LAR and the fitted curves for R, R_L, E and α, based on the polynomial equations derived for W and L (Appendix 1). No statistical comparison can be made between treatments since the fitted curves were based on mean values only.

Figure 12. Comparison of growth parameters of mungbean grown on soils with residues (●) vs soils without residues (o). Units used are:

R -- $mg \cdot g^{-1} \cdot day^{-1}$

R_L -- $cm^2 \cdot dm^{-2} \cdot day^{-1}$

E -- $mg \cdot dm^{-2} \cdot day^{-1}$

LAR -- $dm^2 \cdot g^{-1}$

α -- R/R_L

These are averages across incubation periods.

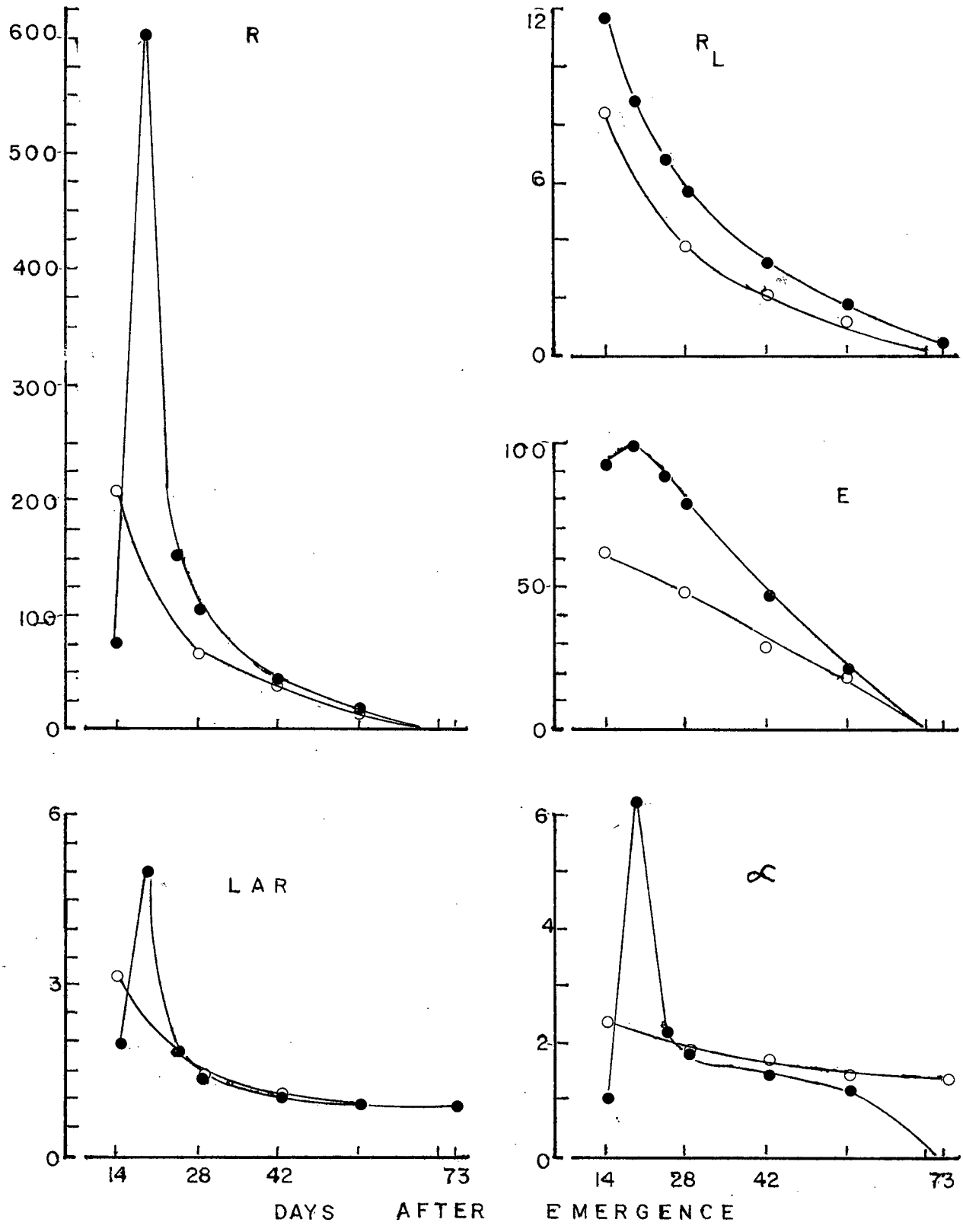

R
600
500
400
300
200
100
0
R_L
12
6
0
E
100
50
0
LAR
6
3
0
14
28
42
73
DAYS AFTER EMERGENCE
∝
6
4
2
0
14
28
42
73

Figure 13. Total dry weight (W), leaf area (W_L), relative growth rate (R), relative leaf area growth rate (R_L), net assimilation rate (E), leaf area ratio (LAR) and α of the succeeding mungbean crop grown on soil containing leaf and root residues after a one-week incubation. Units as for Figures 11 and 12.

Fallow : ● - actual data; o- fitted data; solid line.

Residue : ■ - actual data; □- fitted data; dashed line.

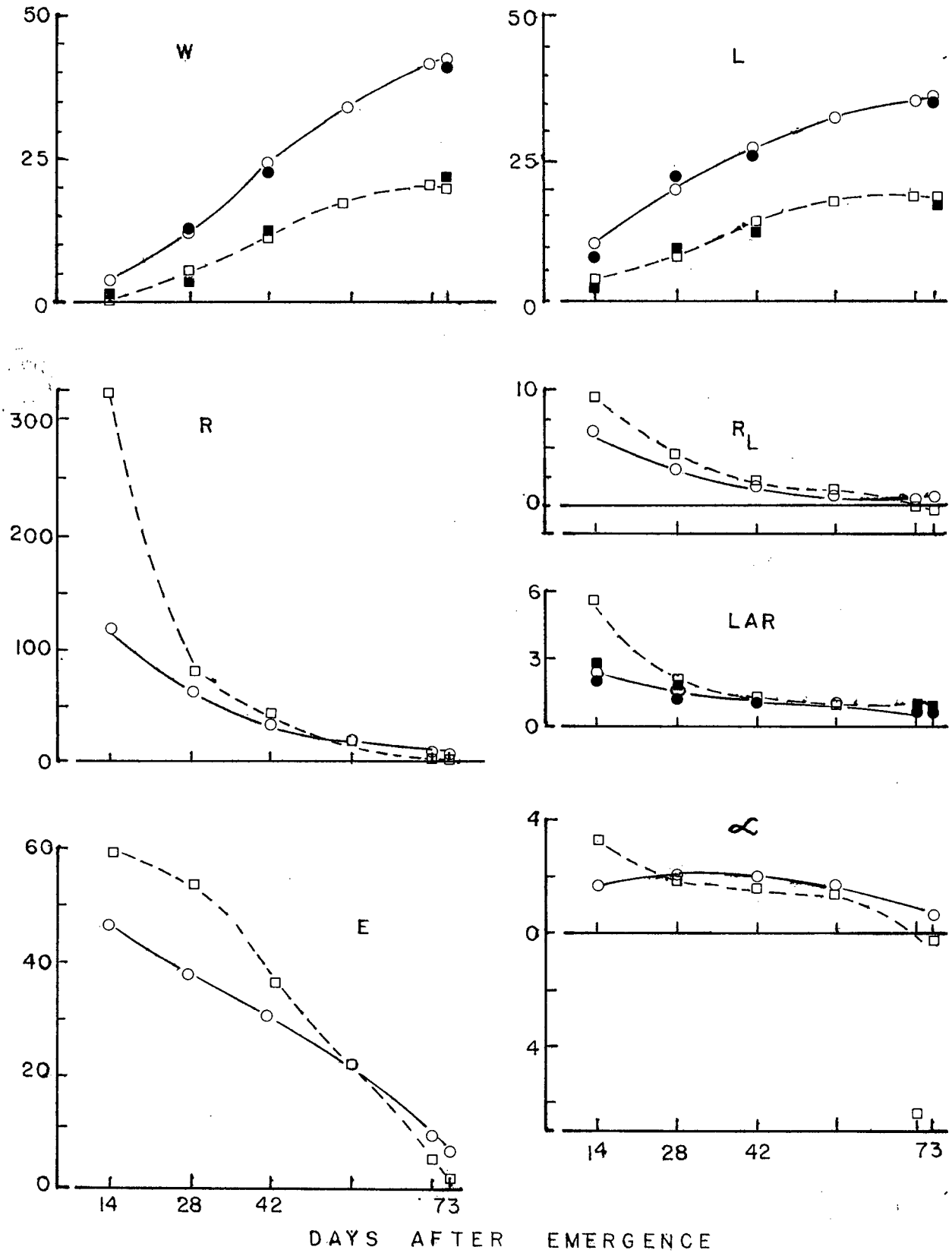
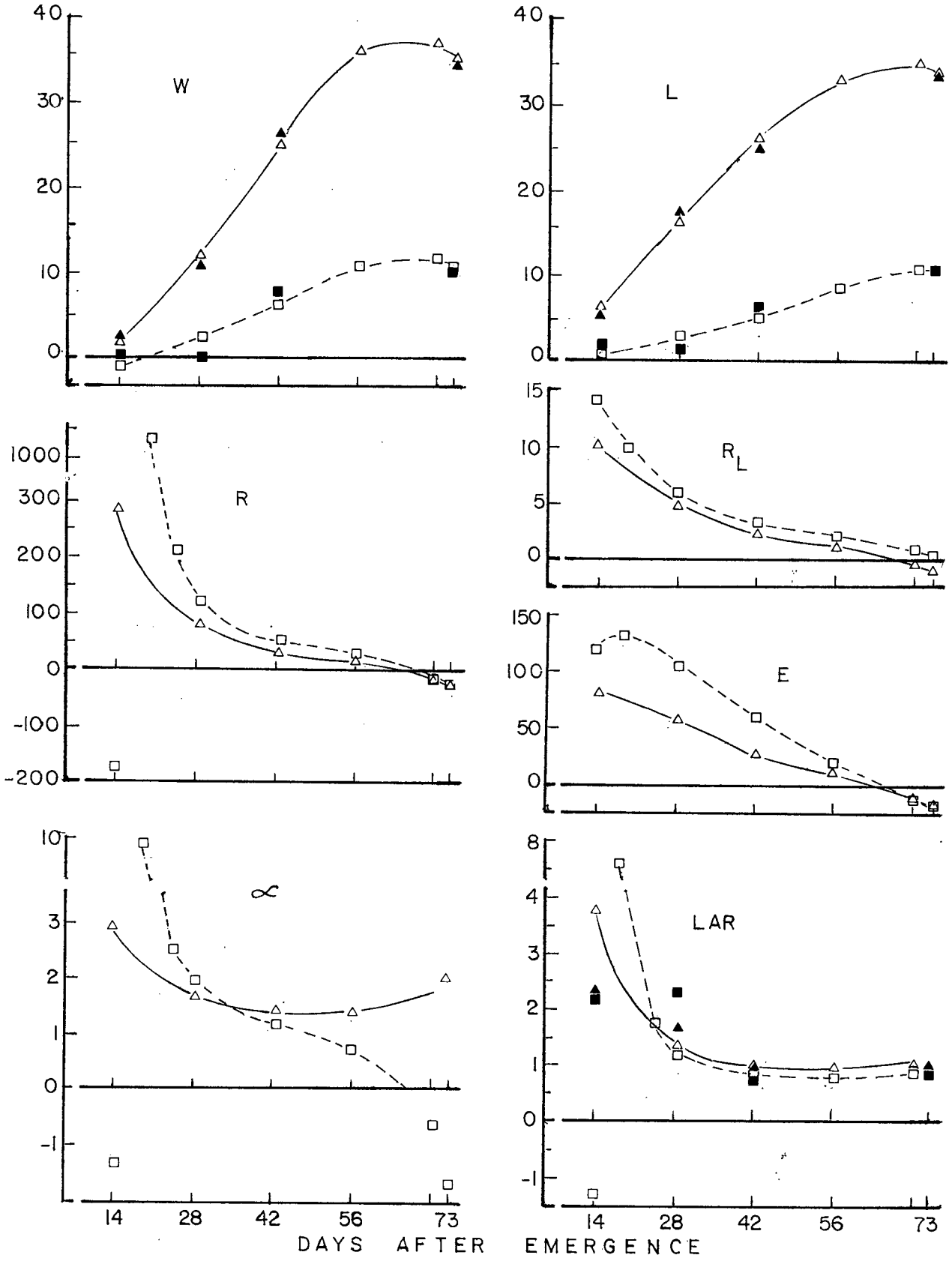

W
L
R
R_L
LAR
E
α
50
25
0
300
200
100
10
6
3
60
40
20
4
14
28
42
73
DAYS AFTER EMERGENCE

Figure 14. Total dry weight (W), leaf area (L), relative growth rate (R), net assimilation rate (E), relative leaf area growth rate (R_L), leaf area ratio (LAR) and α of the succeeding mungbean crop grown on soil containing leaf and root residues after a three-week incubation period. Units as for Figures 11 and 12.

Fallow : ▲- actual data; △- fitted data; solid line.

Residue : ■- actual data; □- fitted data; dashed line.


W
L
R
R_L
E
∝
LAR
40
30
20
10
0
1000
300
200
100
-100
-200
15
5
150
50
8
4
3
2
1
-1
14
28
42
56
73
DAYS AFTER EMERGENCE

A comparison of Figures 13 and 14 reveals that the two sets of control plants behaved similarly. Thus they demonstrated similar overall growth curves for dry weight and leaf area in terms of both form and magnitude. The same is generally true for the derived growth parameters, although there are greater differences revealed with respect to the magnitude of some of the values. This is probably the result of slight differences in the growing conditions to which the plants were subjected, because of the two-week difference in time of seeding. The only parameter showing a markedly different trend over time is α . However, in both sets, the overall trend is for α to decline slightly from a value close to 2, i.e. the demonstration of a shift from a quadratic towards a linear relationship between W and L.

Inspection of Figures 13 and 14 also shows that the enhancement of the derived growth parameters within the early stages of growth caused by residues was greater following 3-week incubation.

Effects of leaf and root residues.

With the effect of combined leaf and root residues amply demonstrated in Experiment 3a, it became of interest to find out which of the two residues was the source of the greater toxicity. With this information, investigation

of the mechanics of phytotoxicity and the indentification of the phytotoxic compounds responsible could be focussed on a specific source.

Experiment 3b was designed to compare the separate effects of leaf residues with those of root residues. The experiment was generally the same as Experiment 3a with the addition of treatments in which only leaf or root residues were incorporated into the soil. Because of an early problem of establishing the plants, which was probably due to using too cold water for watering the pots at the start, some pots had to be discarded. As a result only three samplings were possible, i.e., 30, 52, and 75 days after emergence.

As with Experiment 3a, total dry weights, component weights (leaves, stems, roots and pods), leaf areas, LAR's, LWR's and SLR's were subjected to analyses of variance. Comparisons and contrasts of treatment effects were done in the following manner:

a) with <u>vs</u> without leaf residues

$$\bar{x}_1 = 1/2(T_1 + T_3) \quad \underline{vs} \quad \bar{x}_2 = 1/2(T_4 + T_2);$$

b) with <u>vs</u> without root residues

$$\bar{x}_3 = 1/2(T_2 + T_3) \quad \underline{vs} \quad \bar{x}_4 = 1/2(T_4 + T_1);$$

where T_1 = mean of leaf residue treatment;

T_2 = mean of root residue treatment;

T_3 = mean of leaf and root residue treatment;

T_4 = mean of control

The effects of the mixed leaf and root residues on growth which had been observed in Experiment 3a were again demonstrated and were shown to be more pronounced in the case of the leaf residue treatments than the root residue treatments. Table 8 summarizes the contrast between the treatments with or without leaf residues and treatments with or without root residues on the components of growth and total dry weight. It can be clearly seen that the highly significant effects of residues already presented in Table 6 were mainly due to the presence of leaf residues.

The differences in the effects of the two types of residues on the growth of a succeeding mungbean crop are graphically illustrated in Figures 15 and 16. The primary data are presented in Appendix 5. The magnitude of the effects of the presence of leaf residues on leaf area and the components of dry weight are clearly shown in Fig. 15. In each case the presence of leaf residues results in a decrease in leaf area or weight at each harvest. In relative terms, the greatest reduction is in the weight of roots. However, it should also be noted that, because of the relatively greater effect on total dry weight than on leaf area, or leaf weight, both LAR and LWR are increased slightly but significantly.

With regard to the effects of root residues depicted in Fig. 16, the differences at all harvest dates are reduced to non-significance in relation to the effects

Table 8. Summary of contrasts between treatments with vs without leaf residue and treatments with vs without root residue.

Variables	with vs without Leaf residue	with vs without Root residue	leaf x root Interaction
Total dry weight	**	*	*
Leaf area	**	*	ns
Leaf weight	**	*	*
Stem weight	**	ns	*
Root weight	**	**	**
Pod weight	**	ns	ns
LAR	**	ns	*
LWR	**	ns	**
SLA	ns	ns	ns

** -- Statistically significant at 1% level.

* -- Statistically significant at 5% level.

ns -- Non-significant

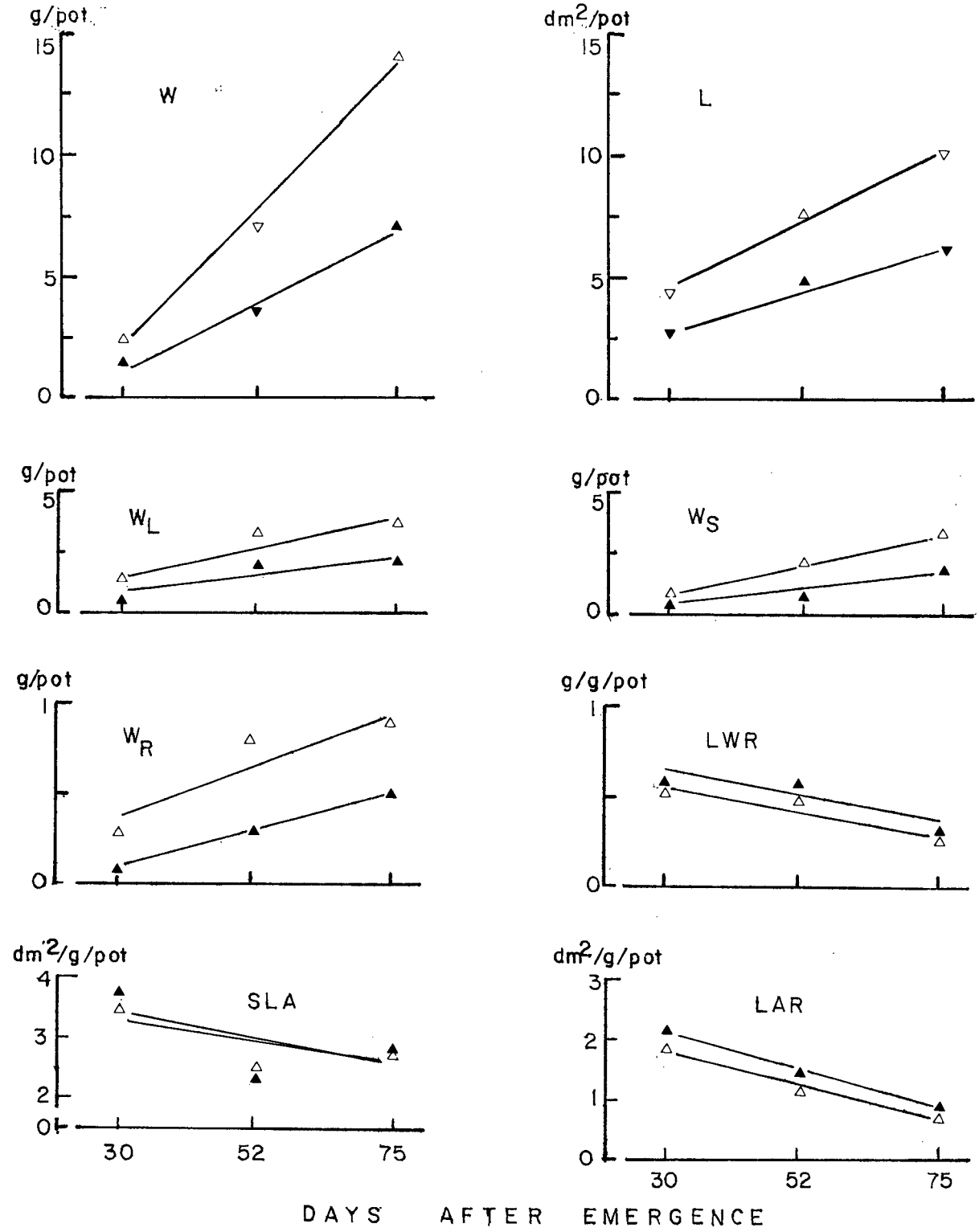


Figure 15. The effect of leaf residue on the total dry weight (W), leaf area (L), leaf (W_L), stem (W_S), root (W_R) weights, leaf weight ratio(LWR), specific leaf area ratio (SLA) and leaf area ratio (LAR).

△- without leaf residue; ▲-with leaf residue. Regression lines for W, L, W_L and W_S are highly significant; the rest are non-significant.

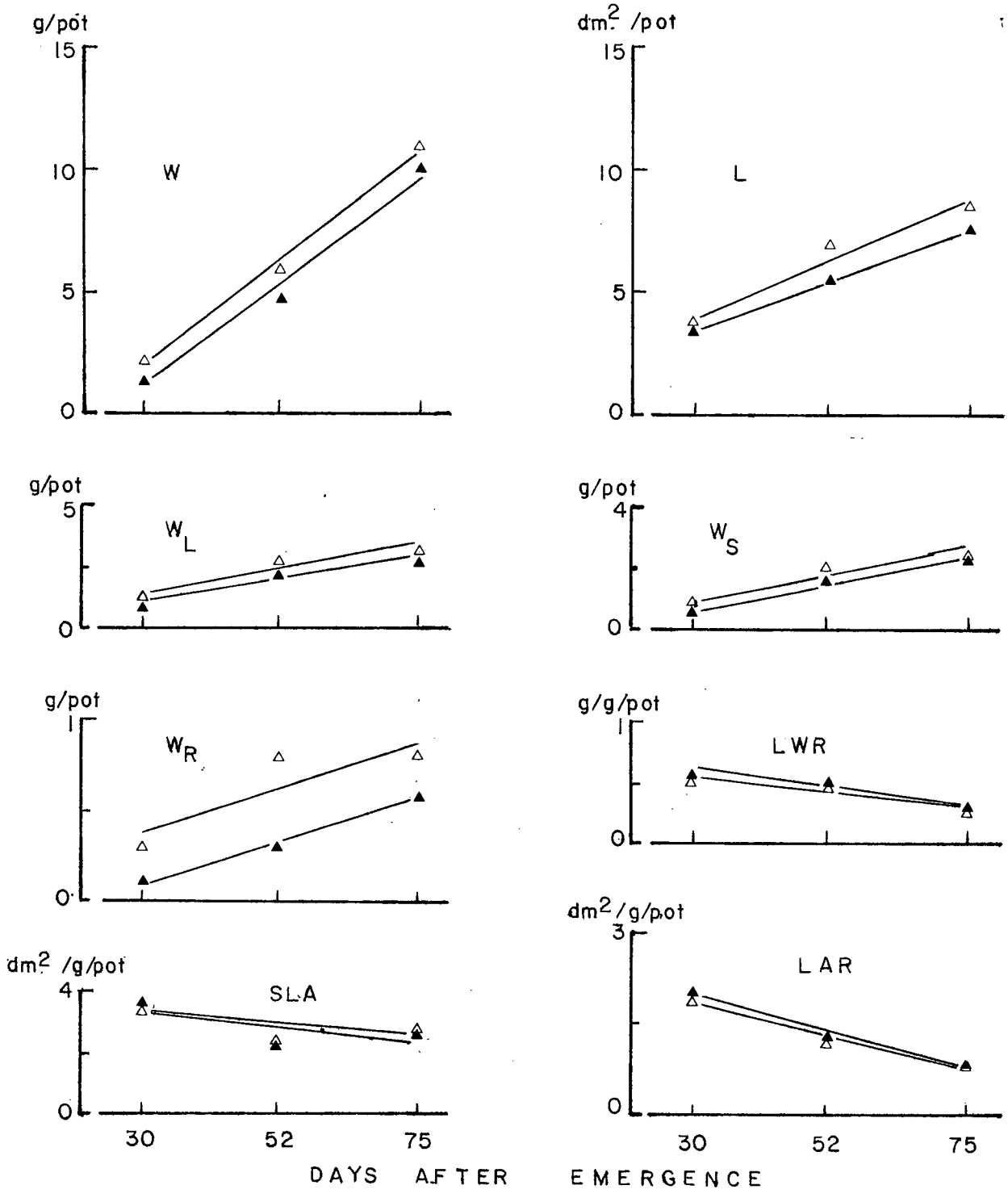


Figure 16. The effect of root residue on the total dry weight (W), leaf area (L), leaf (W_L), stem (W_S), root (W_R) weights, leaf weight ratio (LWR), specific leaf area ratio (SLA), and leaf area ratio (LAR).

△ -without root residue; ▲ -with root residue.
The above regression lines are non-significant.

of leaf residues. The exception is root weight which is again significantly reduced.

Inspection of Table 8 also shows there were several significant interactions between the effects of leaf and root residues, the most significant being those on root weight and LWR.

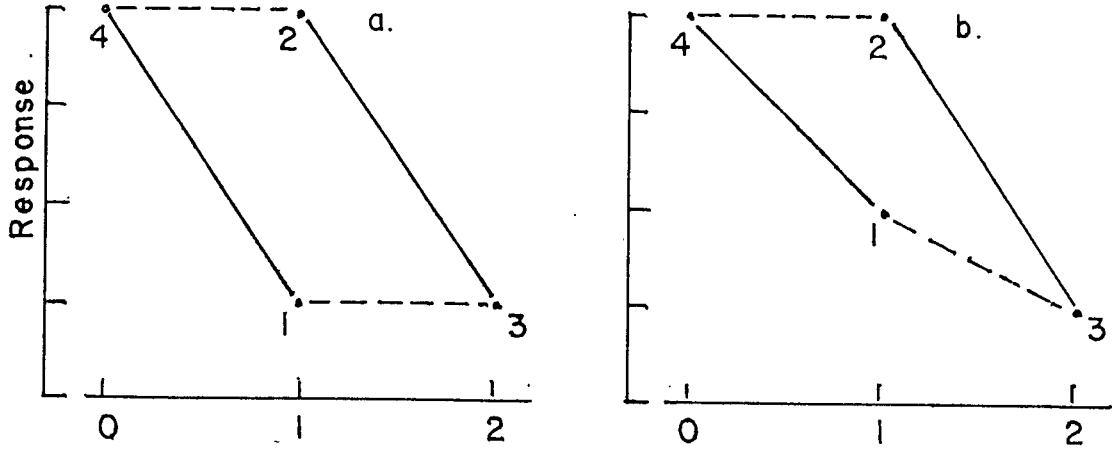
To make the nature and quantitative value of the interactions between leaf and root residues more apparent, use has been made of the diagramatic representation devised by Richards (1941). In such diagrams, the abscissae represent successive increments in the level of one factor or the introduction of additional factors. The coordinates represent the magnitude of the effect on the variable in question.

For example, in this experiment the abscissae for Richards' diagrams for treatment interactions run from zero to 2:

	Level	Leaf residue	
Level		0	1
Root	0	(Treat. 4)	(Treat. 1)
Residue	1	(Treat. 2)	(Treat. 3)

Treatment 4 corresponds to "0", treatments 1 and 2 to "1" and treatment 3 to "2", (abscissae values).

Thus, a and b below are two possible configurations that may result from a 2 x 2 factorial experiment:



In these examples, solid lines reveal the effects of leaf residues, and broken lines those of root residues. The above diagrams illustrate the form and magnitude of interactions. Where there are no interactions between two factors, the diagram takes the form of a parallelogram, with each factor acting independently. The absence of parallelism indicates the interaction between two factors.

Fig. 17 shows the Richards' diagram for the leaf and root interactions indicated in Table 8. It will be noted immediately that there is an absence of parallelism in most of the diagrams which indicates that there are interactions in the effects of both types of residues on the components of growth, although not all reach statistical significance (Table 8), e.g. W_p and L. The highly

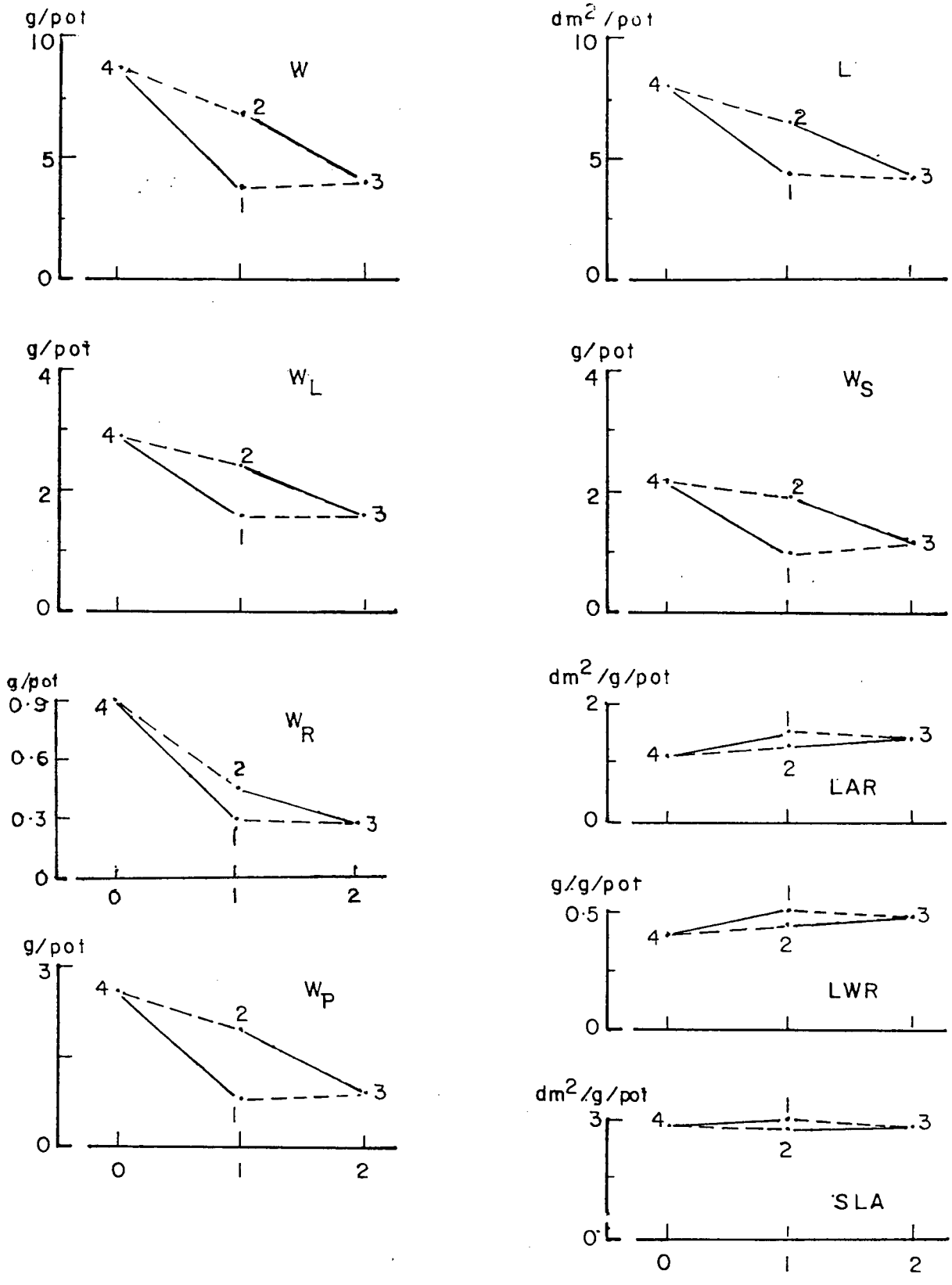


Figure 17. Richard's (factor) diagram of root x leaf residue interactions. Means across sampling dates.

1 - leaf residue
2 - root residue
3 - root leaf residue
4 - control

significant interactions on root weight and LWR show that the presence of either residue alone results in a close to maximal effect. On the other hand, the effects on total weight, the weights of leaves, stems and pods, and on leaf area are clearly shown to be caused by leaf rather than root residues. The relatively greater effect of leaf residue on W than on L and W_L is clearly illustrated in the diagram in which the values for LAR, LWR and SLA with leaf residue are slightly higher from those with root residue.

Figures 18 and 19 present fitted curves for R, E, R_L and α computed from the polynomials derived from the means of W and L (Appendix 1). Although no statistical comparisons can be made, it can be seen that both residue sources cause similar trends in the relative growth rate, relative area growth rate and α . In the case of net assimilation rates, the effect of leaf residues was to cause a reduction throughout the growth period, while with root residues, the rates were maintained throughout and eventually exceeded those of plants grown in the absence of root residues. In general, the values of α show an increase with time, indicating at least a maintenance of the quadratic relationship between W and L. However, it is apparent that the early stimulations of R, E and R_L demonstrated in Experiment 3a were not repeated in Experiment 3b, possibly because of the differences in the season of the year at which the different experiments were conducted.

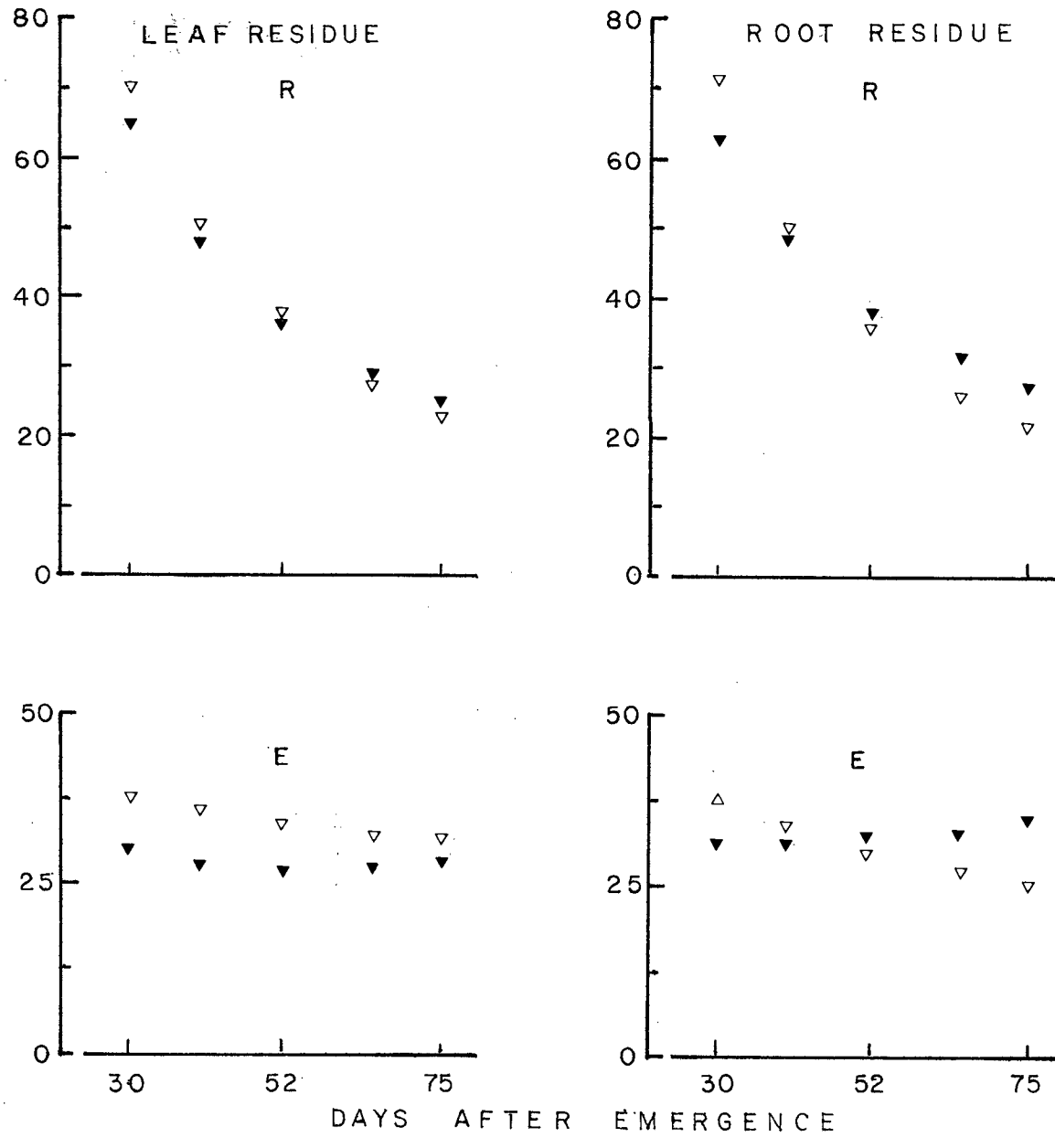


Figure 18. The effect of leaf and root residues on relative growth rate and net assimilation rate of the second crop of mungbeans.

▼ - with residue; ▽ - without residue.

R: mg . g^{-1} . day^{-1}; E: mg . dm^{-2} . day^{-1}

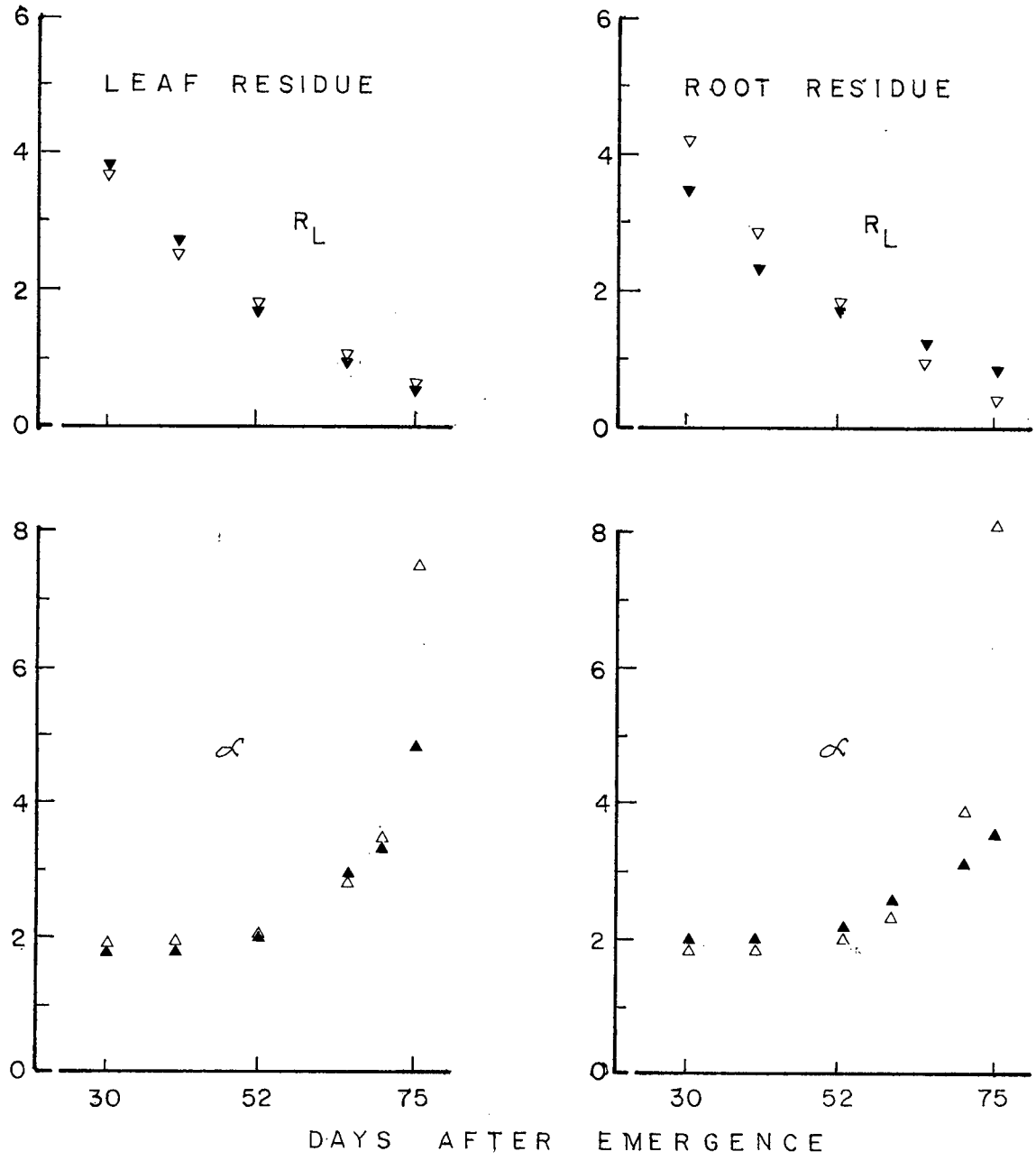


Figure 19. The effect of leaf and root residues on the relative leaf area growth rate and R/R_L ratio of the second crop of mungbean.

▼ - with residue; ▽ - without residue.

R_L: $cm^2 \cdot dm^{-2} \cdot day^{-1}$; α : R/R_L

DISCUSSION

Residue effects. The evidence implicating the leaves as the main source of phytotoxin causing the residue problem in sequential mungbean cropping is quiet clear. The absolute values of total dry weight at any sampling date of plants growing in soil with leaf and root residues (Experiment 3a) is about 50% of the control values in the case of the one-week incubation period and about 40% for the 3-week incubation. Where the effect of leaf and root residues are separated (Experiment 3b), total dry weight attained 43% and 75% of controls for plants grown respectively in soil with leaf- and root-residues.

It has been observed in field experiments that soil with mungbean residues which are kept moist has the greatest effect on the subsequent mungbean crop and that drying of the soil progressively reduces the magnitude of this effect (Runeckles, 1975). Such observation may account for the greater effect of the 3-week incubation since in this study the growing medium was maintained in a moist condition (see Materials and Methods section).

The greater bulk of the leaf dry matter over that of roots, however, may not account for the greater absolute magnitude of the leaf residue effects. The first crop of Experiment 3b had a 3:1 leaf-root weight ratio

on the average. Table 9 presents the weight reduction in percent of control due to leaf, root and leaf plus root residues.

Table 9. Dry weight reduction of mungbean at final harvest, in percent of control, caused by the incorporation in the soil of leaf, root and root plus leaf residues of previous mungbean crop.

Variables	Residues			Toxicity
	leaves (L)	roots (R)	L + R	L/R
	percent			
Total dry weight	53.7	13.8	52.6	3.89
Component weights:				
leaves	43.5	13.2	46.7	3.29
stem	54.1	10.4	50.7	5.2
root	42.6	18.4	50.1	2.3
pods	61.2	15.0	57.5	4.1

In this experiment, it will be recalled that the residues were incubated for twelve days. It will be noted that the leaf-root residue mix caused a reduction of 52.6% on total dry weight which closely corroborates that of the reduction caused by one-week incubation (52.3%) in Experiment 3a. On a land area basis under field conditions, therefore, there are more leaf residues than root residues from the previous mungbean crop. However, assuming that the phytotoxin is evenly distributed in the plant tissues, it is apparent that the leaf residue which is shown to

cause about 4 times more reduction of total dry weight (Table 9) is 12.3% (53.7 - 13.8 x 3) more toxic on a proportionate residue weight basis. Leaf residue caused even more reduction of stem and pod weight, 22.9% and 16.2% respectively.

As to why the leachates of decomposing leaves (Experiment 2) did not show phytotoxicity can only be surmised here. It could possibly be understood once the identity and nature of the phytotoxin is known. There are some potent inhibitors, such as aglycones, which are only very slightly soluble in water (Rice, 1974). It is also possible that the harmful effects of mungbean residues behave similarly to the findings of Patrick et al. (1963) in that root injury to lettuce and spinach seedlings was confined mainly to those parts in direct contact with or in the immediate vicinity of decomposing plant fragments in the soil. Organisms isolated from lesions at the point of injury were found to be mostly nonpathogenic and phytotoxic substances were presumed to have been extracted from plant residues that had decomposed under natural conditions for various periods.

The presence of some phytotoxicity from the roots (Experiment 3b), on the other hand, can be assumed to be attributable to some breakdown products of the root tissues since leachates from the rhizosphere of intact plants (Experiment 1) were not phytotoxic. This may be similar to the case of African marigold *(Tagetes erecta)*,

which contains active nematocides in its roots, but which has failed to yield isolates of the compounds from the exudates of intact roots (Clayton and Lamberton, 1964). The possibility cannot be ruled out, however, that the root exudates may contain compounds which when degraded by microorganisms produce phytotoxins and that the experimental conditions were not favorable for microbial growth.

Since no bioassay was done on leaf tissue extracts, it is not possible to tell whether the phytotoxin is present in the leaf tissues or is only formed and released by decay. There is always the possibility that non-toxic compounds in the plant tissues may be transformed to toxic ones by microbial metabolism. A good example is the case of amygdalin in peach root residue (Patrick, 1955).

Growth parameters. Except for R_L, the derived growth parameters of Experiment 3a show little similarity to those of Experiment 3b. Probably, growth was influenced by season of planting. Experiment 3a, which was planted in summer (July 26, 1978), had the highest dry matter accumulation. Figure 20 presents the extent of reduction of dry matter accumulation due to season of planting (r^2=0.769); the negative correlation (r= -0.877) is significant at the 5% level. As shown in the figure, Experiments 3a and 3b were grown during two distinct seasons where the maximum possible light duration changed from 16 to 13.5 hours and from 10 to 8.2 hours respectively.

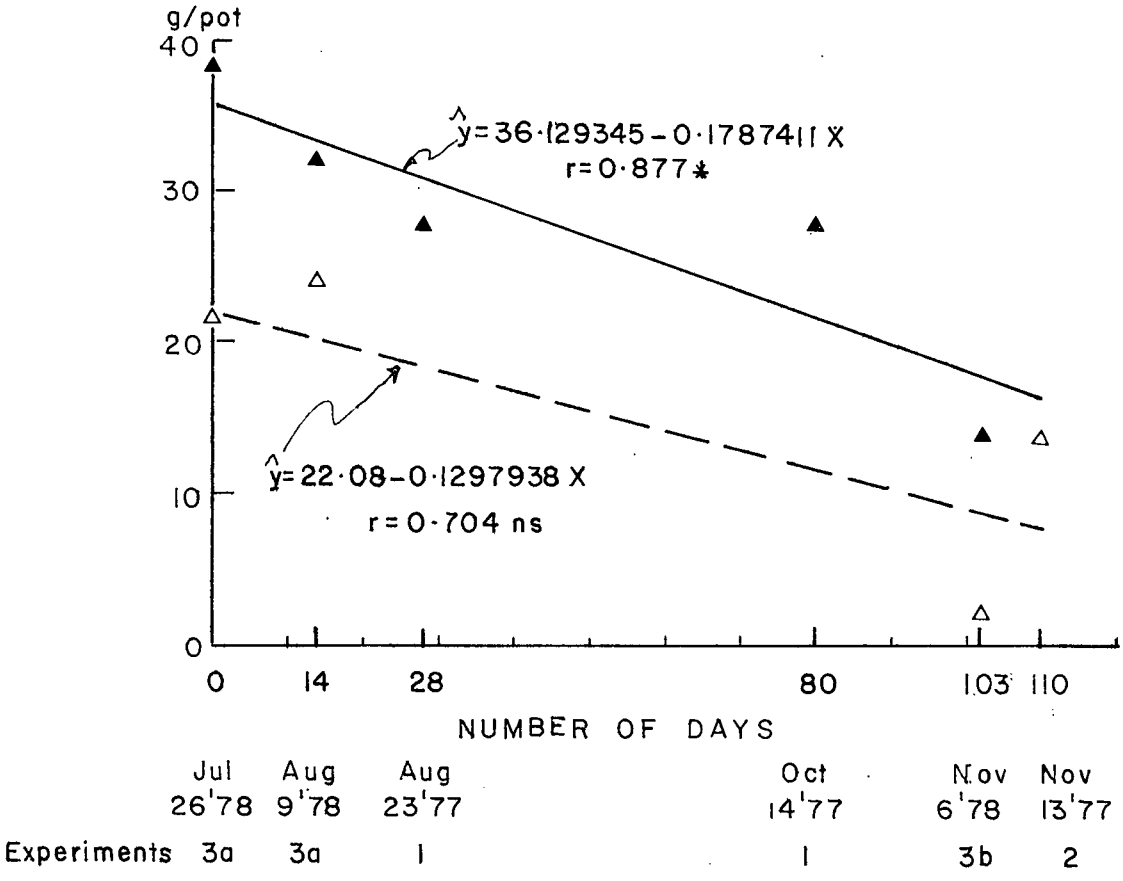


Figure 20. Reduction in dry weight of tops of mungbean as influenced by date of planting. Each observation represents an average of the control treatment.

▲ : harvested at maturity (70-75 DAE)
△ : harvested at reproductive stage (25-35 DAE)

Light quality and intensity under the glass roof during these two periods would have been also different, the later season being more cloudy.

Poehlman (1978) reported little success with mungbean variety field trials grown above 40° latitude, apparently due to the delay in flowering resulting from long photoperiod or poor growth due to the cooler temperature. Mean minimum temperature for productive growth appears to be between 20° and 22°C with the optimum mean temperature in the range of 28°-30°C.

In both Experiments 3a and 3b, temperature was within the above ranges (see Materials and Methods) and apparently, flowering was not affected by light duration since there was no observed change in the days-to-flowering time. This observation suggests that the difference in dry matter accumulation would have been due to the difference in light intensity and spectral distribution. This problem was not anticipated. It was thought that the Lucalox Sodium-vapor lamps, which deliver light in the photosynthetically active region of the light spectrum, were adequate.

Although the growth pattern in Experiment 3b was different from that of Experiment 3a, the reduction in dry matter accumulation due to residue, which in Experiment 3b was clearly shown to be due mainly to leaf residue, is faithfully consistent. The derived growth parameters of the control treatments in Experiment 3a however agree very closely to the ones reported by Tsiung (1978).

There is further similarity between Experiments 3a and 3b. Figures 21 and 22 present the comparative effects of the residue treatments in two Experiments on the partitioning of assimilates over time. These figures summarize the data presented in Appendices 6 through 9. It will be noted that the presence of residues stimulated the accumulation of more assimilates in the leaves at the vegetative stage, i.e., up to 28 days after emergence (Fig. 21). This stimulation was more pronounced as the incubation period of the residue was brought up to 3 weeks. But the increased assimilatory surface, however, did little for the recovery of the plant, inspite of the apparent stimulation of E and R at that period (Fig. 12) since the residue-grown plants attained only about 50% of the total dry weight of control at final harvest. The same stimulated allocation of assimilates to the leaves is shown with leaf residues alone, and to a lesser degree with root residues alone, in Figure 22, which also shows the delay in pod formation among leaf-residue-treated plants which was not observed in Experiment 3a. This delay in pod formation may also be due in part to seasonal effects, as discussed earlier.

The most striking observation in Experiment 3a is the apparent stimulatory effect of residue on the derived growth parameter E particularly at the vegetative stage (Fig. 12). Since E represents the net photosynthetic gain over respiratory loss and may vary according to the magnitude of respiration (Leopold and Kriedemann, 1975),

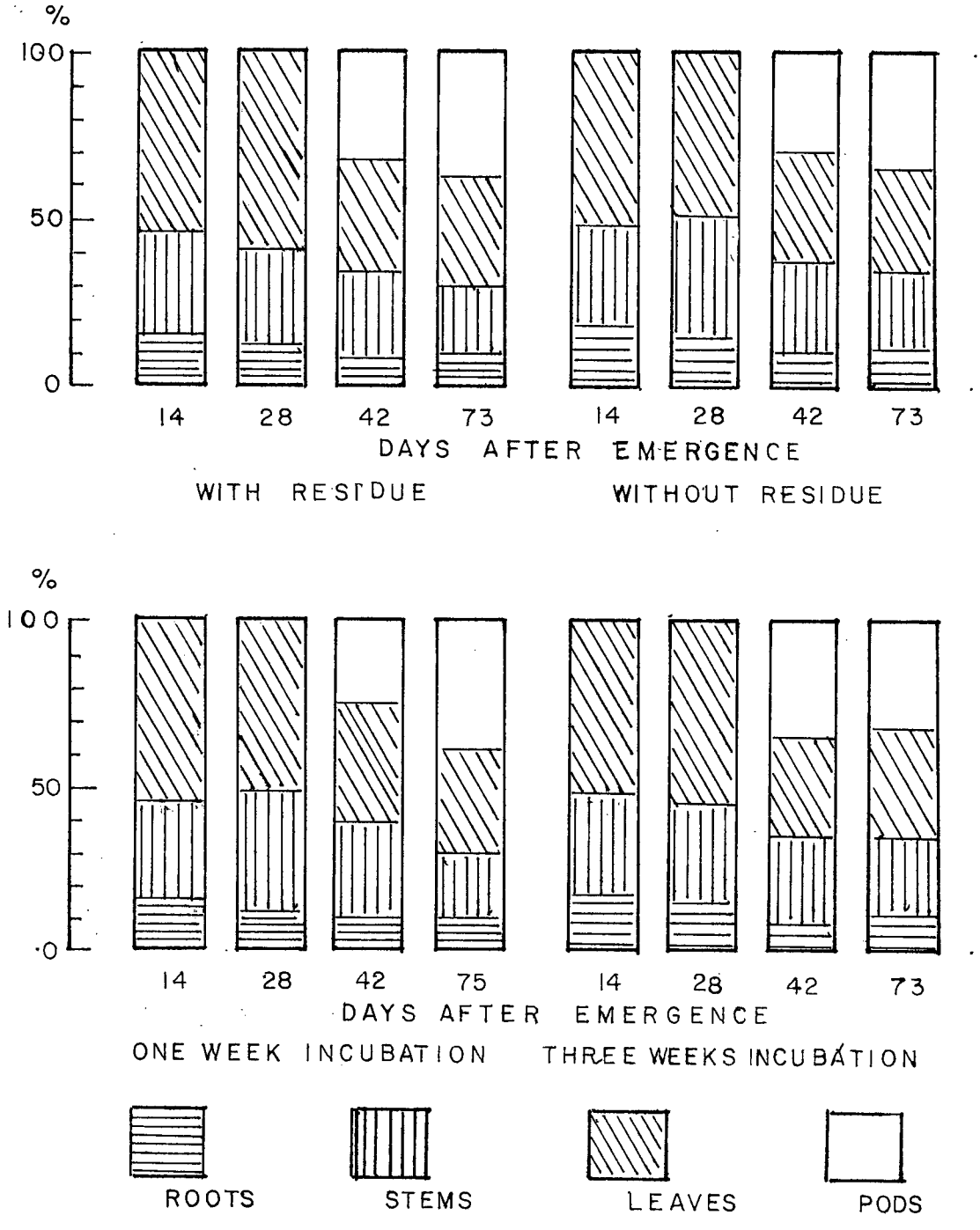


Figure 21. Component dry weights as percent of total dry weight of succeeding mungbean crop as affected by the residue and length of incubation of previous mungbean crop.

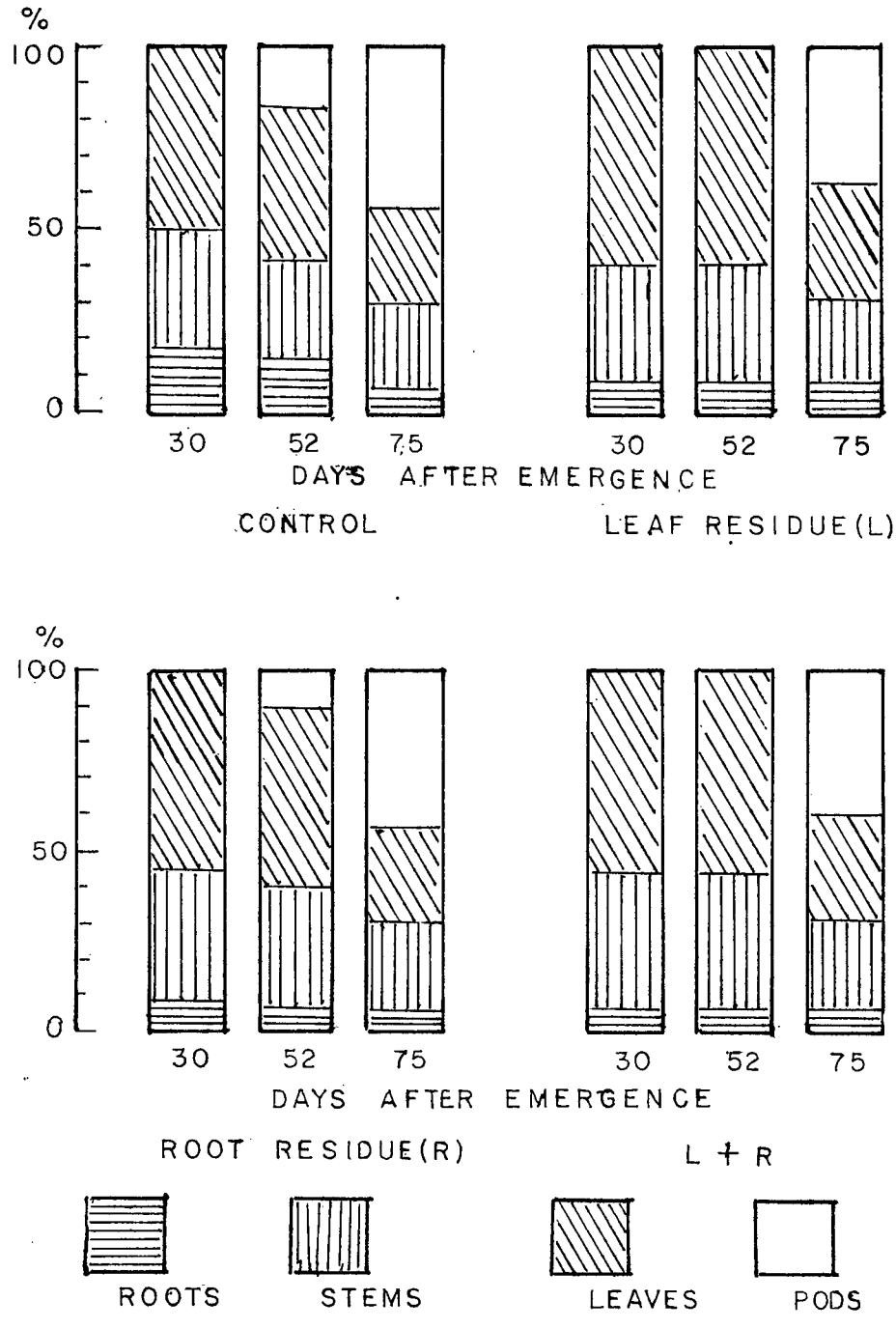


Figure 22. Component dry weights as percent of total dry weight of succeeding mungbean crop as affected by the leaf and/or root residues of previous mungbean crop.

the apparent greater magnitude of the rate of increase of R during the vegetative stage relative to the increase in assimilatory surface (Fig. 12) may account for the greater value for E over this period. This will imply that there was less respiratory loss of assimilates during the period. This may suggest that the phytotoxin from the residue acts as a respiratory inhibitor to growing seedlings after it has caused initial damage during the germination process. It will be recalled that there was more assimilate allocated to the leaves among the residue-grown plants (Fig. 21), thus further inhancing increased net photosynthesis, over those plants grown without residues.

This pattern of effect of stress is similar to that found of water-stressed tomato plants (Gates, 1955) in which, after wilting, lamina weight ratios became higher than those of controls, stem weight ratios became lower and E and R rose above control values.

The present studies are in keeping with the objectives of pinpointing the specific source of phytotoxin from the plant residue of previous crop and describing its effect on the growth parameters of the succeeding crop. The present studies do not permit any elaboration of the plant status earlier than 14 days after emergence. However, the implications of the overall results point out that the effect starts right at the germination process. The status of the plant at the time of sampling (or observation) reflects the cummulative consequence of whatever the plant was subjected to earlier. Hence,

the observed differences in the growth parameters between plants grown in residue-treated soil and those from residue-free soil suggest the need to elaborate the effect observed at the time of emergence as indicated by the conditions of the seedlings (Figs. 2 through 10) of the residue-treated soil.

Obviously, it would be interesting to determine the effect of leaf residue on the germination process, and particularly its effect on respiration. Effects on early stage of growth, such as during elongation of the hypocotyl and tap root development, would also be interesting to know. Information along these lines would be useful in developing bioassay methods for isolated compounds from leaf tissue extracts. Such information would also be useful in developing screening procedures to survey the occurrence of the phytotoxin among mungbean cultivars.

SUMMARY

The purpose of this study was to investigate the effects of a mungbean crop on the growth parameters of a succeeding mungbean crop grown under various conditions of potential transfer and source of phytotoxicants. The mungbean variety, MG50-10a, used in the experiments is high-yielding and is known to have residue problem in a mungbean-mungbean sequential cropping.

The residue problem appeared not to be a simple release of phytotoxin from root exudates of intact plants or of decaying plant materials. The effect is more complex. The main results are summarized below:

1. Root exudates leached from growing plants in sand medium did not show phytotoxicity. This suggests that root exudate per se is non-phytotoxic. However, they may contain compounds which, through microbial metabolism, produce phytotoxins.

2. The residue effect was shown to be dependent on physical contact between subsequent crop roots and residues. Length of decomposition, up to 3 weeks, increased phytotoxicity. Leachate transferred from decomposing residues in sand did not show phytotoxicity.

3. Leaf residues were shown to be more phytotoxic than root residues.

4. Leaf plus root residues were shown to have no additive effect.

5. Residue treatment prevented normal seedling development and residue-grown plants attained about half the total dry weight of controls.

6. Plants in residue-treated soil have more assimilate allocated to the leaves during the vegetative stage, compared to those from residue-free soil. During this stage E, R, R_L and LAR becomes considerably greater than for the controls. Although R_L is increased, which may be due to more assimilates being allocated to the leaves, the greater magnitude of the increase in R over R_L may account for the increase in the value of E. This would be possible if there is a reduction in respiratory losses, which suggests that the residues may be releasing a respiratory inhibitor.

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APPENDIX

Polynomial equations fitted to dry weights and leaf areas and Tables of means of fitted and actual data and other growth parameters of Experiments 3a and 3b.

APPENDIX 1

Polynomial equations fitted to the mean total dry weight (W) and leaf area (L) data on sampling time (t).

Experiment 3a.

Treat. 1 $W = .39941 - .18684t + .016089t^2 - .00013422t^3$

$L = .16887 + .14576t + .007351t^2 - .000080782t^3$

Treat. 2 $W = .21569 + .020066t + .020216t^2 - .0007209t^3$

$L = .50749 + .83727t - .0036837t^2 - .000014149t^3$

Treat. 3 $W = .62291 - .27338t + .015732t^2 - .00013699t^3$

$L = .26105 - .041463t + .0058086t^2 - .000043845t^3$

Treat. 4 $W = .4742 - .389t + .039174t^2 - .00037363t^3$

$L = .19406 + .27297t + .015845t^2 - .00018043t^3$

Experiment 3b.

Treat. 1 $W = .001 + .0012317t + .0012827t^2 - .00000097851t^3$

$L = .001 + .043475t + .0018639t^2 - .000017437t^3$

Treat. 2 $W = .001 + .018902 + .0012173t^2 + .000010828t^3$

$L = .001 + .10865t + .00085193t^2 - .0000085666t^3$

Treat. 3 $W = .001 - .00087638t + .0015323t^2 - .000003547t^3$

$L = .001 + .088115t + .00023259t^2 - .0000051767t^3$

Treat. 4 $W = .001 + .063168t + .0063179t^2 - .000037707t^3$

$L = .001 + .10983t + .0024819t^2 - .000027688t^3$

APPENDIX 2

Actual and fitted data on the changes on mean total dry weights (W) in Experiment 3a.

Treatments	Days After Emergence	Actual (g/pot of 4 plants)	Fitted (g/pot of 4 plants)
1) One week Incubation	14	1.012	0.569
	28	4.254	4.835
	42	11.270	10.989
	73	20.260	20.284
2) Control for Treat. 1	14	3.748	3.987
	28	13.162	12.849
	42	27.818	23.970
	73	42.478	42.466
3) Three weeks Incubation	14	0.776	-0.497
	28	0.626	2.295
	42	7.550	6.743
	73	11.140	11.211
4) Control for Treat. 3	14	2.402	1.681
	28	11.148	12.093
	42	26.016	25.558
	73	35.452	35.487

APPENDIX 3

Actual and fitted data on the changes on mean leaf areas (L) in Experiment 3a.

Treatments	Days After Emergence	Actual (g/pot of 4 plants)	Fitted (g/pot of 4 plants)
1) One week Incubation	14	2.498	3.099
	28	8.698	7.910
	42	12.562	12.943
	73	18.260	18.227
2) Control for Treat. 1	14	8.566	10.453
	28	22.212	19.737
	42	25.914	27.111
	73	35.580	35.478
3) Three weeks Incubation	14	1.664	0.699
	28	1.426	2.692
	42	6.130	5.518
	73	11.080	11.132
4) Control for Treat. 3	14	5.514	6.238
	28	16.860	15.911
	42	25.394	25.854
	73	34.018	33.980

APPENDIX 4

Means of total dry weight, leaf area, dry weights of leaves, stems, roots and pods. (Weights are expressed in g/pot of 4 plants and leaf area in dm^2/pot of 4 plants).

Experiment 3a.

Variables	Days After Emergence 14		28		42		73	
			1 week incubation/control					
Total dry weight	1.01	3.75	4.25	13.16	11.27	23.82	20.26	42.48
Leaf area	2.50	2.57	8.70	22.21	12.56	25.91	18.26	35.58
Leaf dry weight	0.60	1.99	2.27	6.76	4.29	8.52	6.98	12.86
Stem dry weight	0.27	1.12	1.50	4.71	3.10	6.40	3.82	8.08
Root dry weight	0.14	0.64	0.48	1.69	0.98	2.28	2.10	3.86
Pod dry weight	-	-	-	-	2.90	6.62	7.36	17.68
			3 weeks incubation/control					
Total dry weight	0.78	2.40	0.63	11.15	7.55	26.02	11.14	35.45
Leaf area	1.66	5.51	1.43	16.86	6.13	25.39	11.08	34.02
Leaf dry weight	0.41	1.27	0.40	5.56	2.14	8.08	3.70	11.84
Stem dry weight	0.26	0.72	0.13	4.20	1.78	7.36	2.10	9.10
Root dry weight	0.12	0.41	0.09	1.39	0.49	2.20	1.12	3.55
Pod dry weight	-	-	-	-	3.14	8.38	4.22	10.96

APPENDIX 5

Means of total dry weights, leaf area, dry weights of leaves, roots, and pods. (Weights are expressed in g/pot of 4 plants and leaf area in dm^2/pot of 4 plants). Experiment 3b.

Variables	Treatments			
	Leaves	Roots	Root & Leaves	Control
		30 DAE		
Total dry weight	1.166	1.956	1.258	2.774
Leaf area	2.212	3.797	2.713	4.781
Leaf weight	.688	1.086	.708	1.378
Stem weight	.380	.710	.460	.888
Root weight	.098	.160	.090	.508
		52 DAE		
Total dry weight	3.396	5.798	3.600	8.498
Leaf area	4.850	6.751	4.563	8.532
Leaf weight	2.004	2.878	2.038	3.520
Stem weight	1.118	1.888	1.314	2.270
Root weight	.274	.412	.248	1.282
Pod weight	-	.620	-	1.426
		75 DAE		
Total dry weight	6.896	12.834	7.058	14.894
Leaf area	6.390	9.327	5.732	10.518
Leaf weight	2.202	3.384	2.078	3.900
Stem weight	1.596	3.118	1.714	3.480
Root weight	.566	.804	.492	.986
Pod weight	2.532	5.548	2.744	6.528

APPENDIX 6

Root/Weight Ratios

Experiment 3a

Treatment	Days After Emergence 14	28	42	73
1) 1 week incubation	.14	.11	.09	.10
2) Control (1)	.17	.13	.10	.09
3) 3 week incubation	.15	.14	.06	.10
4) Control (3)	.17	.12	.08	.10

Experiment 3b

Treatment	Days After Emergence 30	52	75
Leaf residue (L)	.08	.08	.08
Root residue (R)	.08	.07	.06
R & L	.07	.07	.07
Control	.18	.15	.07

APPENDIX 7

Stem/Weight Ratios

Experiment 3a

Treatment	Days After Emergence			
	14	28	42	73
1) 1 week incubation	.27	.35	.28	.19
2) Control (1)	.30	.36	.27	.19
3) 3 week incubation	.33	.21	.24	.19
4) Control (3)	.30	.38	.28	.26

Experiment 3b

Treatment	Days After Emergence		
	30	52	75
Leaf residue (L)	.33	.33	.23
Root residue (R)	.36	.33	.24
R & L	.37	.37	.24
Control	.32	.27	.23

APPENDIX 8

Leaf/Weight Ratio (W_L/W)

Experiment 3a

Treatment	Days After Emergence			
	14	28	42	73
1) 1 week incubation	.59	.53	.34	.34
2) Control (1)	.53	.51	.36	.30
3) 3 week incubation	.52	.63	.28	.33
4) Control (2)	.53	.50	.31	.33

Experiment 3b

Treatment	Days After Emergence		
	30	52	75
Leaf residue (L)	.59	.59	.32
Root residue (R)	.55	.50	.26
R & L	.56	.57	.29
Control	.50	.41	.26

APPENDIX 9

a) Pod/whole plant weight ratio.

Experiment 3a

Treatment	Days After Emergence 42	73
1) 1 week incubation	.26	.36
2) Control (1)	.28	.42
3) 3 weeks incubation	.42	.38
4) Control (3)	.32	.31

b) Pod/whole plant weight ratio

Experiment 3b

Treatments	Days After Emergence 52	75
Leaf residue (L)	0	.37
Root residue (R)	.11	.43
R & L	0	.39
Control	.17	.44