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Project leader:	Dr J.F. Smith, Horticulture Research International, Wellesbourne, Warwick CV35 9EF (ceased employment 31st Jan, 1997),
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Project Co-ordinator:	Peter Woad
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CONTENTS

PRACTICAL SECTION FOR GROWERS

Objectives and background

While mushroom yield per unit weight of spawned compost has increased by over 50% in the last 20 years, no mushroom strain on the market today will produce successive flushes in equal proportion. This is not surprising since mushroom mycelium has first to establish itself in its substrate and then secondly, during substrate utilisation and fructification, it produces 'staling products' which gradually pollutes the environment in which it lives. For large commercial farms operating a 4 flush strategy, up to 70% of the total crop can be produced in the first two flushes. This can create picking problems (mushrooms distort due to competition for bed space) and invariably quality loss which ultimately results in revenue loss.

In recent years, much has been published at HRI on how the mushroom degrades its substrate, yet little progress has been made in the commercial exploitation of this knowledge. While the mushroom totally colonises its substrate prior to fructification, carbohydrates so essential for fruitbody development are utilised from the cellulolytic fraction of the compost. It is now known that the mushroom concentrates its cellulolytic activity (e.g. extracellular endo-cellulase) to the uppermost layer of the compost first, thereby minimising the distance carbohydrates have to be translocated to the developing fruitbodies. On depletion of carbohydrates in the uppermost region of the compost, mushroom mycelium then progressively concentrates its activity on lower undegraded layers until ultimately, the total volume of substrate has been utilised. This study reports on the placement of biodegradable barriers in colonised compost layers to temporarily restrict or reduce the passage of nutrients to the developing fruitbodies in the early flushes. In theory, a physical barrier, albeit a temporary one, would create for a short period, two distinct compost layers, one operating in a reproductive mode whereas the isolated lower layer would remain in a vegetative mode whilst the barrier remained intact. If a biodegradable barrier could be found which could survive 3-4 weeks when placed between two spawn-run compost layers, it is very likely that first flush and possibly the second flush would be reduced whilst later flushes would be increased.

The use of both paper and uncoated cellophane films as temporary barriers are examined in this study. Both are available in sheet/roll form that can be easily inserted into present mushroom growing systems without major disruption of present technology and their cost is generally minimal. As the materials under test are also biodegradable, they will disappear during the cropping cycle and therefore offer no problem in the dispersal of spent materials.

The ultimate objective of this work is to produce similar weights of good quality mushrooms throughout successive flushes. Uniformity of cropping, if it could be achieved, would be a major step forward in mushroom growing technology and it may also be of great advantage to robotic cropping machines that are presently being developed that normally find closely packed mushrooms difficult to harvest.

Summary of Results

This preliminary investigation of 6 months, was to determine as quickly as possible, suitable biodegradable barriers (both paper and cellophane) that when placed in colonised compost layers would delay the passage of nutrients to the developing fruitbodies during the first and possibly the second flush. Their effect on flush pattern and overall yield were carefully recorded in the three small scale experiments where small volumes of compost (400/450g) were used in transparent plastic containers.

The first experiment was designed to test two types of paper (a) Kimwipe - an absorbent porous dual layer paper and (b) Newsprint - a strong inexpensive waxed wood pulp paper. All the paper barriers were positioned midway in the container such that equal weights of compost were above and below the barrier. These containers were cased with a standard casing mixture, and the effect of the barrier placement on yield and flush pattern was monitored and the results compared with a control treatment without a barrier. In all the treatments where barriers were placed midway in the compost layer, increases in number and yield of mushrooms were recorded on the first flush. The greatest increase in yield was obtained in the treatment where Kimwipe, the dual layer porous paper, was employed. These results were unexpected as the placement of the barrier was intended to suppress first flush yield and not increase it. It was also evident that in both the Newsprint treatments, yield increases also occurred in the second flushes. In all cases where a biodegradable barrier was placed to form two separate layers, increases in yield above the control treatment, were achieved over the 4 flushes. Anastomosis of mushroom mycelium between the separated layers was clearly observed during the later stages of the first flush suggesting that it took between 3-4 weeks for the barrier to degrade.

In the second experiment, paper barriers were compared with a range of uncoated cellophane films of varying thickness (300-600µm). Unlike the first experiment, the results recorded from identical paper barrier treatments differed in that decreases in the first flush yield was achieved in comparison to the control. While the second flushes were virtually unaltered, notable improvements in yield occurred in later flushes to give an improved spread of mushrooms over the four flushes. There were also reductions in first flush yield when cellophane barriers were used with improvements recorded on the second flushes. Generally third and fourth flush yields were greater in barrier treated composts, but over the four recorded flushes it was notable, as in the first experiment, that in all composts receiving a barrier, total yield was improved. It is not easy to explain the differences in first flush yields recorded in experiments 1 and 2 when paper barriers were used, but it must be remembered that no two composts are the same and while spawn run compost was used in both experiments the degree of substrate colonisation after 14 days may have differed.

In the final experiment, the paper type Kimwipe and the 300 µm cellophane film were chosen as barriers, but additional placements of barriers both closer (one third down) and deeper (two thirds down) to the fruiting surface were tested. As with previous experiments, a treatment where placement of the barrier midway between compost layers was also included. Also included in the treatments was an additional control treatment which consisted of only 50% of the compost used in the standard control. This was included to confirm published findings that the biological efficiency (yield of mushrooms per unit weight of substrate) increases as a compost layer becomes thinner.In this experiment, it was quite clear for the first time that when either a paper or cellophane barrier was placed (one third down) such that 66% of the compost was temporarily partitioned off, a significant reduction in first and second flush yields were achieved. Placement of a barrier, whether paper or cellophane, midway between two compost layers in this experiment had marginal effects of first and second flushes but the third flushes were improved upon. Possibly the most interesting results to date were achieved when the barriers were placed such that 33% of the compost was temporarily partitioned off (two thirds down). For both paper and cellophane barriers, two very high yielding flushes were achieved with a significant improvement in the third flush and as found with the previous 2 experiments, mushroom yield over 4 flushes was, in the majority of cases, greater than the standard control.

It was also confirmed in the final experiment, that in the second control treatment, where only half of the compost was used compared to the standard control, the biological efficiency or yield of mushrooms per unit weight was increased in the thinner layer. Separation of compost layers using paper barriers, albeit temporary, creates a thinner compost layer initially for the mushroom to draw its nutrients. It is highly likely that by placement of barriers as described in this brief report, the biological efficiency in the uppermost layer is improved upon. When the barrier breaks down and reconnection of the layers is completed extracellular enzyme activity is then focused on the lower layer. The overall result is an improvement in yield over the total cropping period.

The initial objective of this study was to make successive flushes more uniform, and while dramatic changes in first and second flush pattern can be achieved using both paper and cellophane barriers at different depths, the use of a single barrier may not give the desired control to produce three or four equal weight flushes. Perhaps the most stimulating result to come from this brief study, is that flush patterns can be dramatically changed at little cost and inconvenience, and improvements in overall yield can be achieved when the mushroom is forced to focus its activity on a particular section of the compost.

Advice to Growers.

Advice on the use of biodegradable barriers would be premature at this early stage of the work. It must be remembered that this study was performed in small containers and the findings of this report first need to be confirmed on a larger scale. It has become quite clear, that to suppress first flush yield, the barrier placement has to be closer to the fruiting surface than was first thought. With strong evidence of yield improvement due to barrier placement alone, a follow up study at a semi-commercial level is urgently required, not only to confirm these exciting findings but to develop a method of cropping where the mushroom grower is always in control.

Science Section

The use of biodegradable barriers within conventional growing systems to unify the production of mushrooms per unit bed of cropping surface throughout successive flushes (HDC contract M28)

J.F. Smith

(Formerly of Horticulture Research International, Wellesbourne, Warwickshire)

Introduction

Mushroom yield per unit weight of spawned compost has increased two fold in the last 20 years, and mushroom growers now regard yields in excess of 250 kilos per tonne of compost as normal. This improvement in yield, together with the use of high density composts, has meant that mushrooms, as they develop, compete for bed space especially during the first and second breaks. Many growers today, especially those growing on aluminium shelf beds, operate a three flush strategy and depending on strain, competition for bed space can be a problem on either the first or second break. After two high yielding flushes, a decline in yield for the third break can be expected, but as the mushrooms are then more evenly distributed on the bed, harvesting becomes easier and the quality of the harvested mushroom is generally improved. For those farmers operating a four flush strategy, over 70% of their mushrooms are normally picked in the first two breaks and quality can sometimes suffer. Much has been learnt in recent years on how the mushroom, utilises carbohydrates from the cellulolytic[AU1] fraction of the composted substrate. It has been quite clearly shown by work pursued at HRI (Smith, 1984; Smith et al 1989; Smith, 1994) that while totally colonising its substrate, mushroom mycelium utilises carbohydrates in the uppermost layer first and then as this layer is depleted, nutrients in the lower layers are utilised. The commercial objective of this work is to investigate methods whereby the flow of nutrients to the developing fruitbodies is controlled in order that more uniform flushes of good quality mushrooms are achieved throughout 3 or maybe 4 flushes. This report investigates the placement of a range of biodegradable barriers in compost beds to create isolated zones, thereby temporarily reducing the availability of nutrients to the developing mushrooms during the vigorous early flushes.

Materials and Methods

Experiment 1.

The first experiment was conducted using two types of paper (a) Paper Towelling (Kimwipe) - an absorbent porous dual layer paper and (b) Newsprint - a strong inexpensive waxed wood pulp paper. Plastic containers measuring (10 x10 x 15cm deep) and capable of holding 400g of prepared compost were used all experiments. For the preliminary investigation, 14 day spawn compost was used and the following four treatments were chosen.

1. Control - each container containing 400g of spawned compost
2. Kimwipe - placed midway in each replicate such that 200g compost was above and 200g compost was beneath barrier
3. Newsprint - as above
4. Newsprint - as above but paper moistened on placement (this treatment was included to test whether a moistened barrier would enhance extracellular enzyme production (cellulases) accelerating breakdown).

Prepared containers (5 replicates for each treatment) were randomly placed in a Fisons Controlled Environment cabinet and the cabinet maintained at 25^{0}C for 7 days to allow re-establishment of mushroom mycelium. All containers were cased to a depth of 2cm with a proprietary mixture of peat and lime and then maintained at 21^{0}C for 7 days to encourage a rapid penetration of the casing layer. On appearance of surface stroma, the cabinet temperature was reduced to 18^{0}C and maintained at this throughout the cropping period. To obtain an accurate assessment of yield differences between treatments, mushrooms were harvested with a clean stipe intact (Biological Yield).

Experiment 2.

In this experiment it was decided to compare the use of paper barriers with **Courtaulds Uncoated** cellophane (cellulose) barriers. Prior to setting up this experiment, a preliminary test was set up to determine the biodegradability of 5 cellophane films of varying thickness (300µm, 325µm, 400µm, 500µm and 600µm). Using 100cm petri-dishes, discs of the above cellophane films were cut and placed midway between two spawned compost layers of 0.5cm thickness. Three replicates were set up for each film type and the film was observed over 3-4 weeks.

On determining the suitability of film types, a 14 day spawned compost was used as with the previous experiment, and the same quantities of compost were placed above and below the barriers. In this experiment, and an identical environmental protocol was followed. In this experiment 5 cellophane films were compared with the two types of paper barrier i.e. Kimwipe and Newsprint. As with the previous experiment, 5 replicate containers were set up for each treatment, all pots were fully randomised within the growth cabinet and mushrooms were harvested until four flushes had been recorded.

Experiment 3.

The knowledge gained on yield changes associated with barrier type and placement in the experiments 1 and 2 were used to design the final experiment. In this experiment 450g of spawned compost was used per container and both paper and cellophane barriers were tested at different depths. The full details of the barrier and placement treatment are as follows:-

1. Control - 450g of spawned compost
2. Control - 225g of spawned compost
3. Kimwipe - 150g above; 300g below
4. Kimwipe - 225g above; 225 g below
5. Kimwipe - 300g above; 125g below
6. Cellophane (300μm) - 150g above; 300g below
7. Cellophane (300μm) - 225g above; 225g below
8. Cellophane (300μm) - 300g above; 125g below

An additional compost control (2) was included in the treatments to measure the biological efficiency of a thinner layer in comparison to the standard control.

As with Experiment 2, 5 replicate pots of each treatment were fully randomised within the growth cabinet, and mushrooms were harvested until 4 complete flushes had been recorded.

Results

Experiment 1

In all 3 treatments where barriers were placed midway in the compost layer, increases in number and yield of mushrooms were obtained on the first flush. The greatest increase in yield was obtained in the treatment where Kimwipe, the dual layer porous paper, was employed (Figure 1). These results were unexpected as the placement of the barrier was intended to suppress first flush yield and not increase it. It was also evident that in both the Newsprint treatments, yield increases also occurred in the second flushes. Overall, increases in yield above the control treatment, between 10 and 13% were achieved over the 4 flushes when a biodegradable barrier was placed in the compost. The greatest improvement on yield was achieved in the un-moistened Newsprint treatment. Photographic evidence of anastomosis of mushroom mycelium occurring between compost layers during the later stages of the first flush (Plate 1) would suggest that biodegradability of these layers took 3 to 4 weeks. On examining the barriers after completion of four flushes it was quite evident that very little of the paper barriers remained and anastomosis or mycelial re-connection of the layers had been restored ..

Experiment 2.

Preliminary Experiment

The preliminary study of cellophane film types sandwiched between two spawned compost layers can be summarised in Table 1.

Cellophane Barrier	14 Days	17 days	24 days
300µm	loss of elasticity	partly dissolved	well degraded - anastomosis well established
325µm	loss of elasticity	part dissolved	notable degradation - some anastomosis
400µm	some loss of elasticity	loss of elasticity but intact	membrane remaining intact- some softening
500µm	some loss of elasticity	loss of elasticity but intact	membrane remaining intact- some softening
600µm	no notable loss of elasticity	very little loss of elasticity	membrane remaining intact- little degradation

It was quite clear the 300µm cellophane membrane degraded the quickest, with loss of elasticity evident after 14 days. After 17 days this cellophane membrane was partly dissolved allowing anastomosis of mycelium to re-occur and similar observations were made for the 325 µm film. After 24 days both these films were well degraded but for the thicker layers, membranes remained intact although some loss of elasticity was evident. Because of the wide range of biodegradability experienced in this test it was decided to use all the film types in the second experiment.

The results obtained in Experiment 2 from the identical paper barriers treatments used in Experiment 1, differed in that decreases in first flush yield were achieved in comparison to the control treatment. While the second flushes were virtually unaltered, notable improvements in yield occurred in flushes 3 and 4 when a paper barrier was inserted.

In all cases where a cellophane barrier was inserted, there was a reduction in first flush yield in comparison to the control treatment yet an improvement in yield for the second flush was obtained. Generally, third and fourth flush yields were greater in the barrier treated composts, and as found in the first experiment, increases in total yield over the 4 recorded flushes were achieved in all composts receiving a barrier treatment.

Experiment 3.

In the final experiment, the paper type Kimwipe and the 300 µm cellophane film were chosen as barriers, but additional placements of barriers both closer (one third down) and deeper (two thirds down) to the fruiting surface were tested. As with previous experiments, a treatment where placement of the barrier midway between compost layers was also included. Also included in the treatments was an additional control treatment which consisted of only 50% of the compost used in the standard control. This was included to confirm that the biological efficiency increases as a compost layer becomes thinner.

The standard control (Treatment 1), as found in experiment 2, gave its highest yield in the second flush and flushes 3 and 4 were considerably reduced. Treatment 2 (the 50% control treatment) gave similar yields in flushes 1 and 2 but as expected yield per unit weight of substrate was far greater than that achieved in the standard control.

In this experiment, it was quite clear for the first time that when either a paper or cellophane barrier was placed (one third down) such that 66% of the compost was temporarily partitioned off, significant reductions in first and second flush yields were achieved. Possibly the most interesting results to date were achieved when the barriers were placed such that 33% of the compost was temporarily partitioned off (two thirds down). For both paper and cellophane barriers, two very high yielding flushes were achieved with a significant improvement in the third flush.

As found with the previous 2 experiments, mushroom yield over 4 flushes was significantly improved above the standard control (with the exception of treatment 6 - cellophane one third down), when a barrier was placed within the compost to temporarily partition off layers of compost.

Conclusions

Attempts to date to control flush pattern have been mainly environmental (Flegg, 1980; Love *et al*, 1986) although physical attempts to reduce pinning in the first flush by raking (ruffling) has been practised for many years (Flegg, 1967; Vedder, 1977). This report discusses the first attempt to be made at controlling flush sizes by insertion of biodegradable barriers in the colonised compost layer. To gain first hand knowledge of the behaviour of such barriers, totally colonised compost was used in all these small scale experiments.

It has been known for sometime (Smith, 1984: Smith et al, 1989: Smith 1994) that mushroom mycelium, while totally colonising its substrate, exhausts organic materials closest to the fruiting surface first and then utilises nutrients in a downward manner. The objective of this preliminary study was to identify biodegradable barrier types that could be placed within the colonised compost to temporarily partition off nutrients to the developing fruitbodies in the early flushes. It was assumed, that if this could be achieved, flushes 1 and 2 would be reduced and then on anastomosis of the two compost layers, flushes 3 and 4 would be increased unifying the flush pattern.

The results obtained from the first experiment made it perfectly clear that such theory is further complicated by the fact that the biological efficiency (weight of mushrooms per unit weight of compost) increases as a colonised compost layer becomes thinner. Flegg and Ganney (1973) in their studies on the cropping of mushrooms on shallow layers of compost demonstrated that compost layers between 2.5 and 5.0 cm can produce significantly higher yields than compost layers of 12.5 cm thickness. In Experiment 1, the placement of a biodegradable barrier midway within the compost bulk actually improved the yield of the first flush. The reasoning behind this increase could therefore be explained solely by the improvement in biological efficiency created by the thinner layer, especially as the lower layer of compost was totally isolated for around 4 weeks before the barrier degraded. The second flush weights were also increased above the control but the most intriguing result was that in all treatments receiving a barrier, total yields over the four flushes were significantly improved. It is highly likely that in the isolated uppermost layer, extracellular enzyme production is more focused and not complicated by zonal enzyme production as shown by Smith (1994) in deep compost columns.

Experiment 2 was designed to confirm the findings of the first experiment while testing alternative cellophane barriers. Unlike the first experiment, when paper barriers were employed, first flush yields were not increased above the control treatments, second flush figures were very similar but third and fourth flushes were notably improved. Significant increases in overall yield above the control treatment were once again apparent when a barrier was employed. The incorporation of cellophane barriers was also a little disappointing as it was expected that wide differences would be apparent considering their biodegradability rates demonstrated in the preliminary assessment. Generally first flush yields decreased with the increasing thickness of cellophane film, and in all cases where a cellophane film was used as a barrier, second flush figures were improved. In most cases third flush figures were also greater than the control treatment. As found with the paper barriers, when cellophane films were used, there was once again an overall improvement in total yield

Experiment 3 was designed to answer some of the inconsistencies appearing in the previous experiments while testing different placements of the barrier. To reduce the number of treatments it was decided to use Kimwipe as the paper barrier and the thinnest cellophane film i.e. 300 μm. A second control treatment, or a container using only half the weight of compost used in the standard control, was also included in the experiment to confirm the fact that thinner layers are more biologically efficient. As shown in the results (Figure 4), the half control treatment produced 29% more mushrooms per unit weight of compost than the standard control. This demonstrated that the thinner isolated layer, is contributing greatly to the improvement in yields experienced in the first flushes when a barrier is used. In this experiment, placement of paper or cellophane barriers one third and two thirds of the way down the compost bulk were also tested. When barriers were placed only a third of the way down in the compost first flush yields were significantly reduced, second flush yields were slightly reduced and an improvement in yield was experienced in the third flush. Possibly the best results obtained with regard to yield, were achieved when barriers were placed two thirds of the way down in the compost as first, second and third flushes were improved upon in comparison to the standard control. For the paper and cellophane barriers, overall increases in yield of 18.4% and 10.0% were achieved (Figure 4).

It is quite apparent from these trials that the placement of biodegradable barriers within compost layers can significantly change the flush pattern and guaranteed improvements in mushroom yield can be achieved. This overall boost in mushroom yield was unexpected as the commercial objective of this work was to unify flush sizes and to some extent this was achieved with definite improvements in 3rd and 4th flush sizes. It is quite clear that further work needs to be done on barrier types, compost placement and insertion time to improve further on these findings. It must also mentioned that these trials were performed with small volumes of compost (400-450g) and more consistent results may be achieved if larger volumes of compost were used e.g. experimental cropping trays capable of holding 50Kg. In all the experiments described in this paper, spawn run compost was used to ensure consistency between replicate treatments. Further experiments need to be performed where biodegradable barriers are placed in compost at the time of spawning as such an operation would be easier to perform at a commercial level. The idea of encapsulating nutrients in cellophane or paper films, to be introduced at the time of spawning, may be another approach at improving later flushes while overcoming the physical problem of barrier insertion.

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Plate 1. Reconnection of mushroom mycelium (anastomosis) between separated layers occurring at the time of the first break of mushrooms. (n.b. thick rhizomorph developing at the reconnection point).

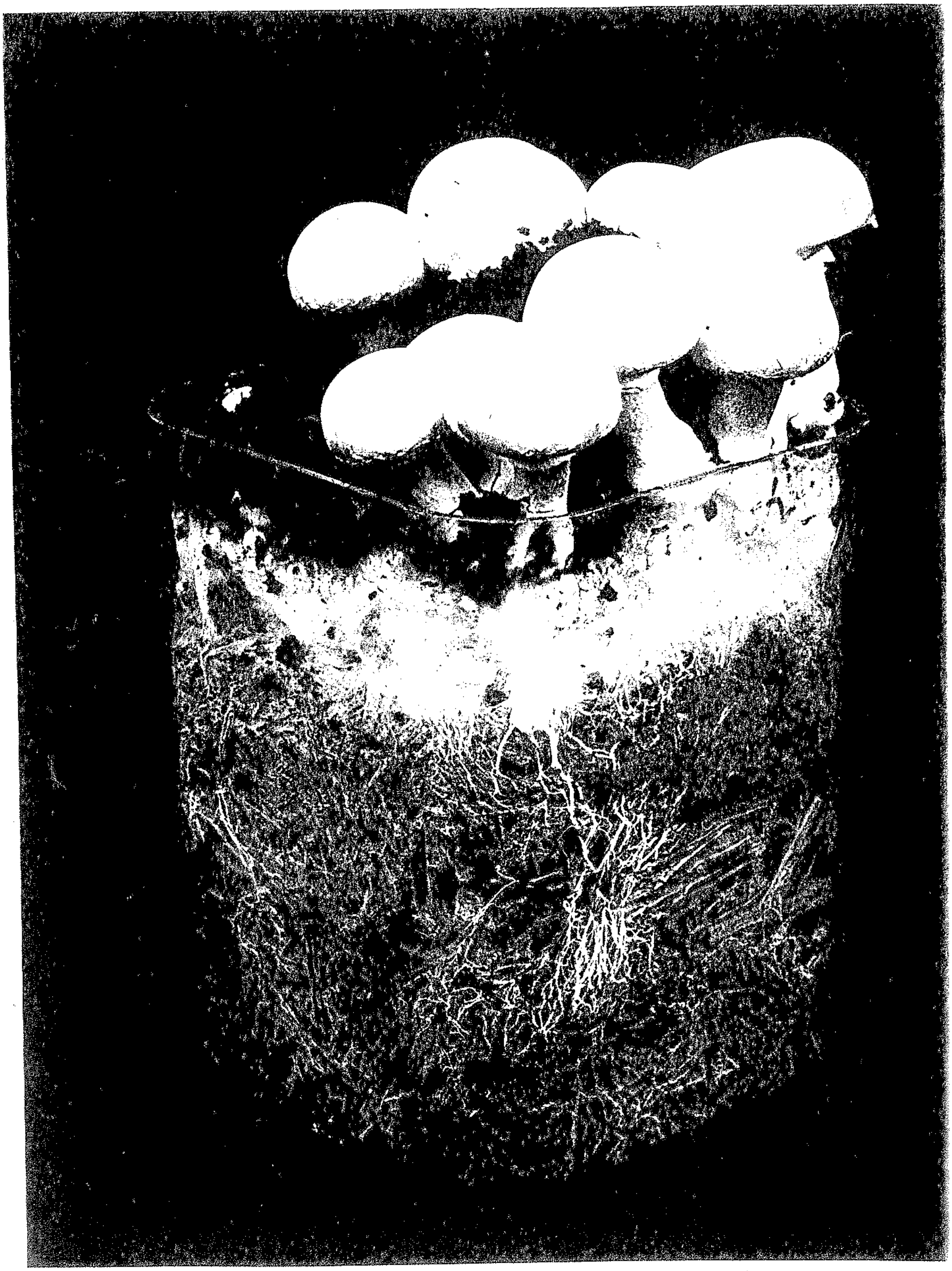


Figure 1. Effect of barrier treatments on mushroom numbers per flush

Experiment 1

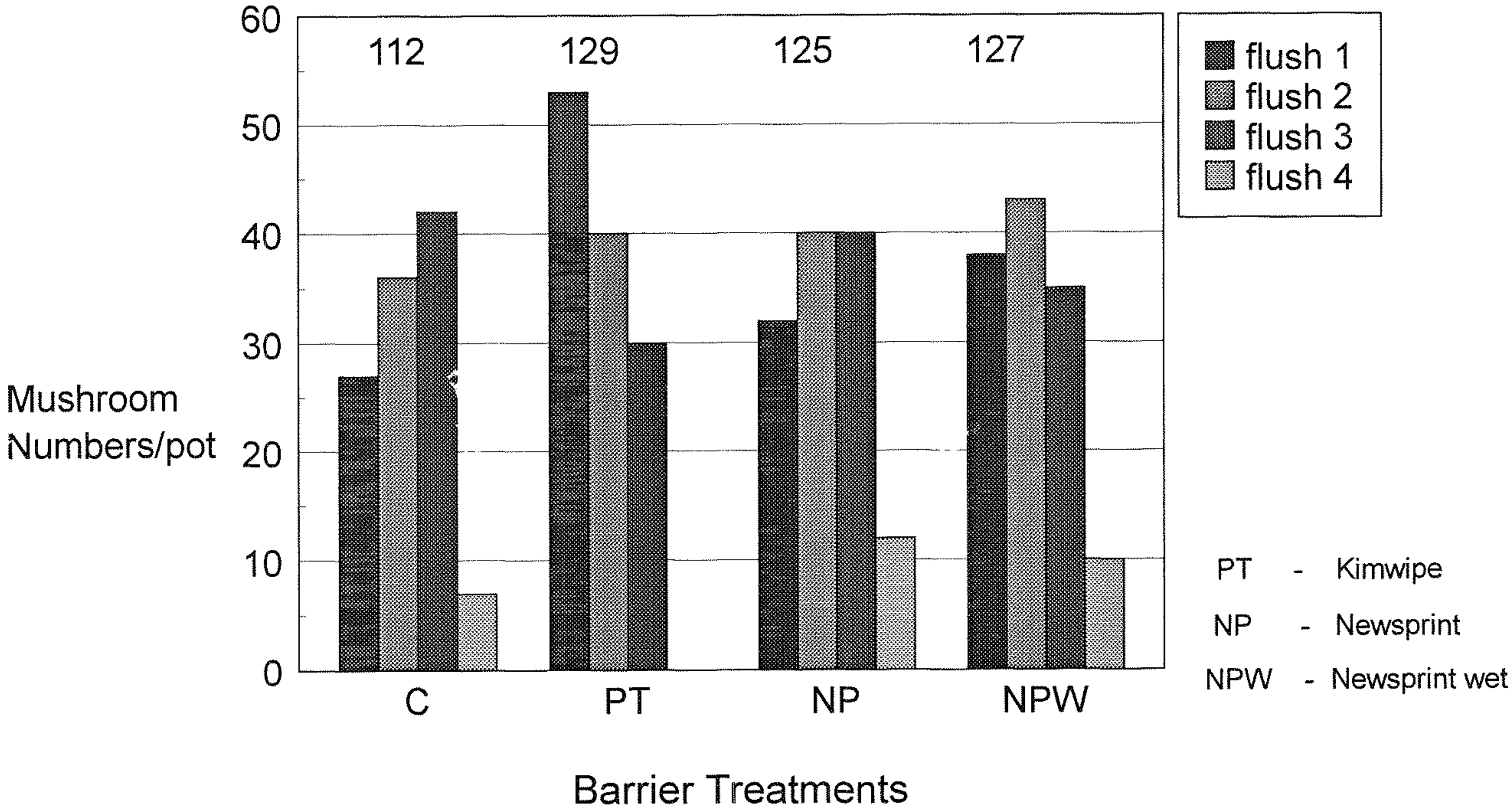


Figure 2. Effect of barrier treatments on mushroom weights per flush
Experiment 1

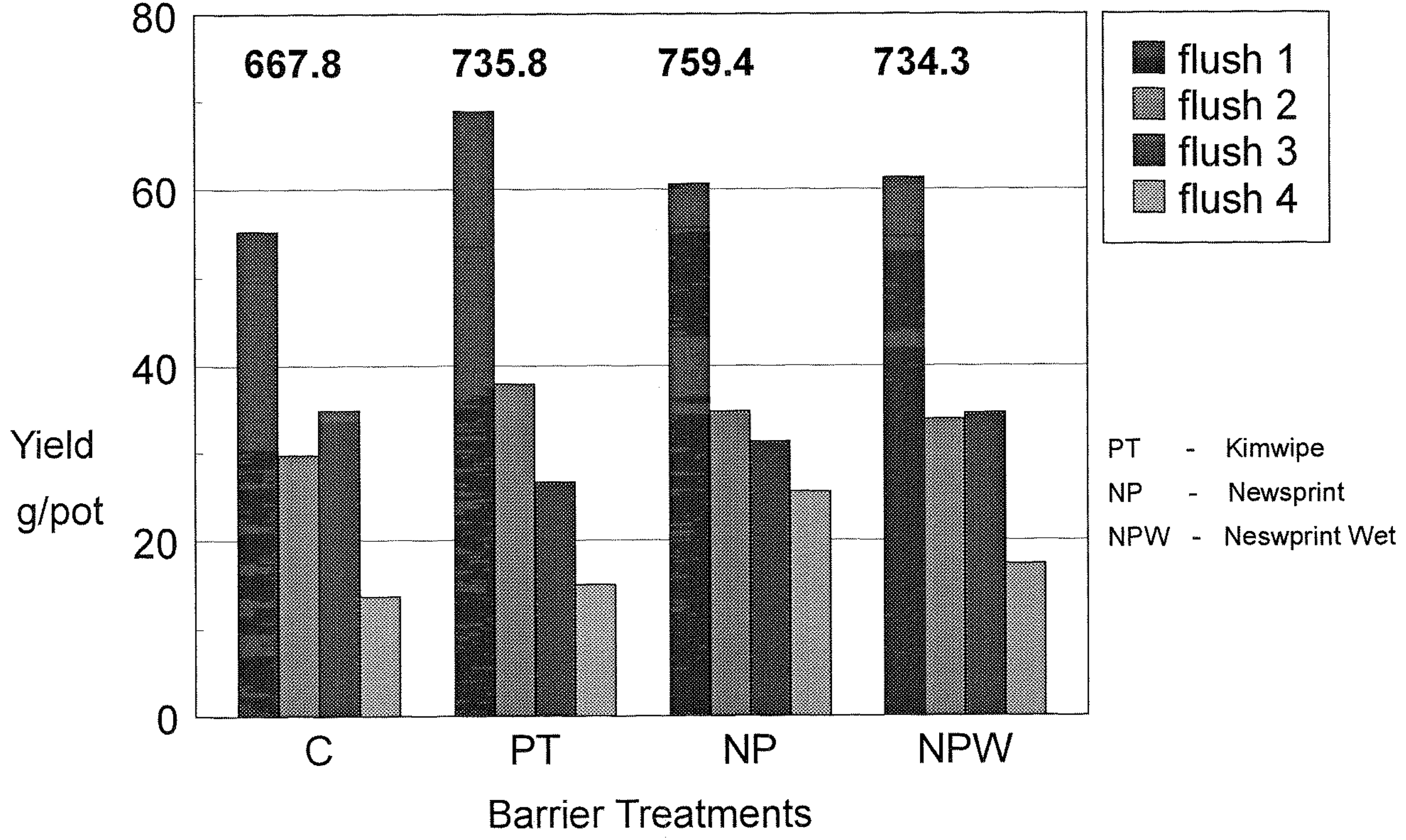
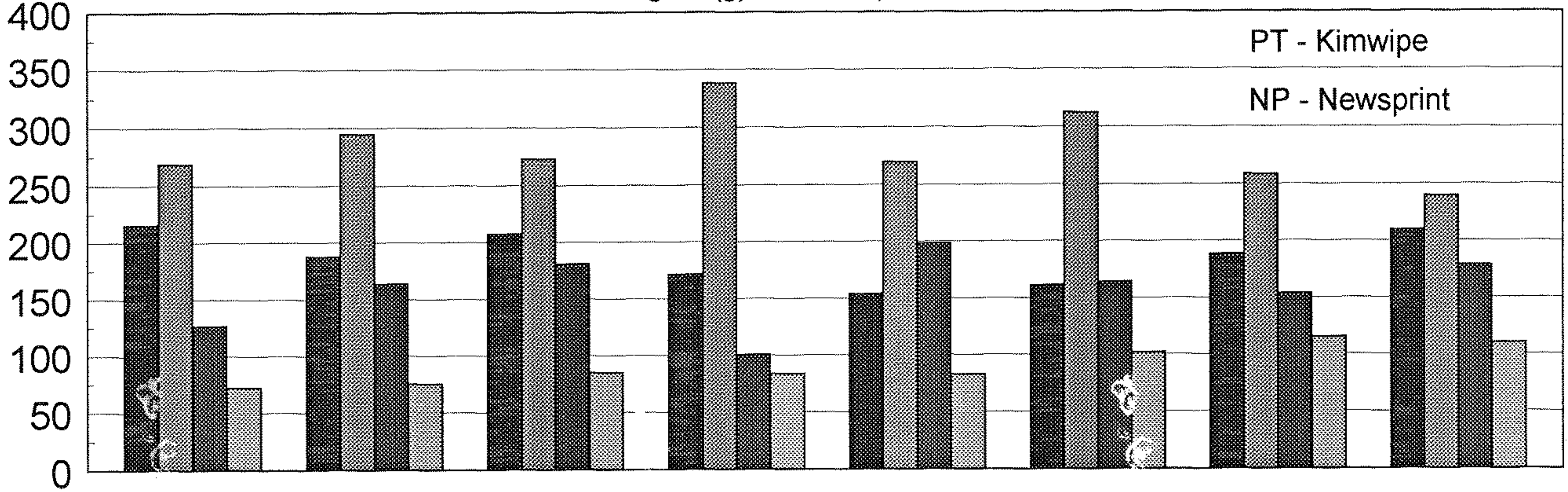


Figure 3. Effect of cellophane and paper barrier placement on mushroom yield per flush

(Barriers placed half way down compost - 200g compost per layer)

Experiment 2

Total weights (g) from 5 replicate containers

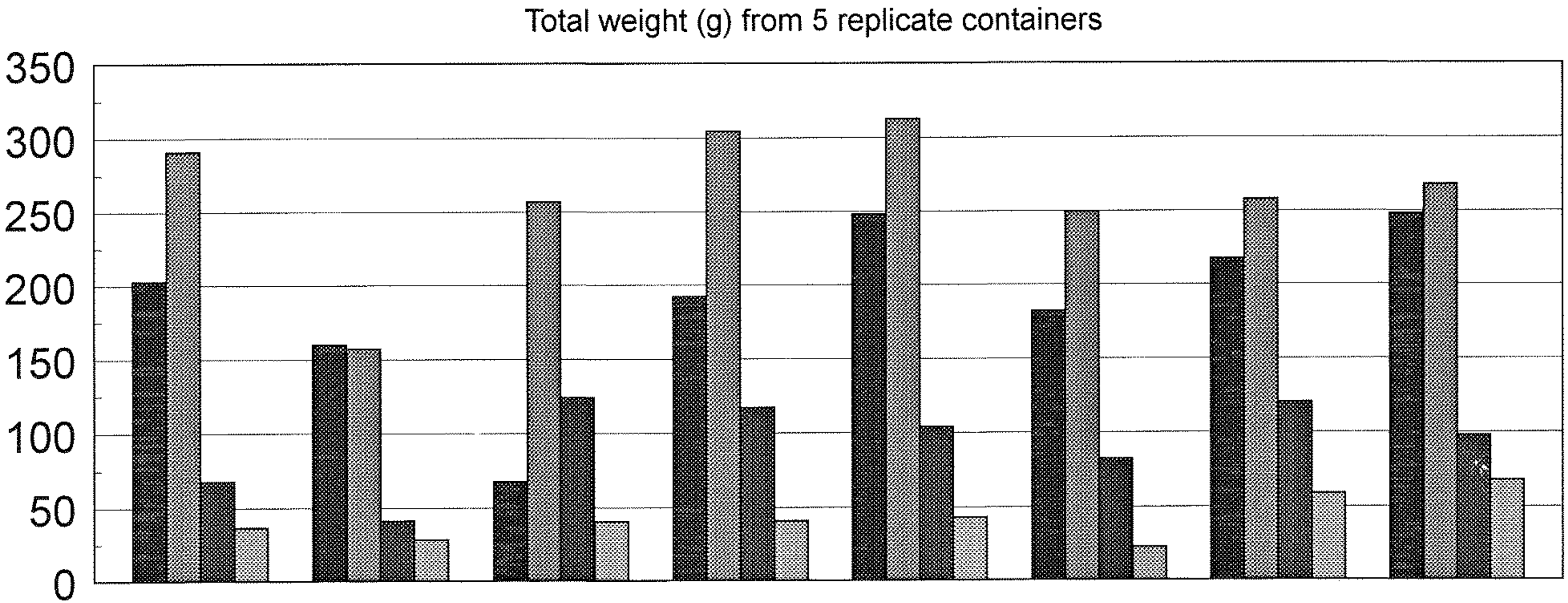


	Control	300	325	400	500	600	PT	NP
Flush 1	216.1	187.7	207.4	171.7	153.9	161.4	188.6	209.7
Flush 2	268.9	294.6	272.9	337.9	269.8	312.5	258.9	239.4
Flush 3	126.3	163.4	181.0	100.3	198.7	164.0	153.4	179.0
Flush 4	71.9	75.1	84.7	83.4	82.7	101.8	115.2	110.2
Total Wts	683.2	720.8	746.0	693.3	705.1	739.7	716.1	738.3

Figure 4. Effect of cellophane and paper barrier placement on mushroom yield per flush

(Barriers placed one third, half way and two thirds down in the compost - 450 g compost per container)

Experiment 3



	Control	Half Control	Paper 33% Above	Paper 50% Above	Paper 66% Above	Cell 33% Above	Cell 50% Above	Cell 66% Above
Flush 1	203.0	160.2	67.5	192.3	248.1	182.3	217.3	248.0
Flush 2	291.2	157.3	256.7	304.5	313.0	249.7	257.8	268.2
Flush 3	67.5	41.3	124.0	116.9	104.3	82.5	120.5	97.9
Flush 4	36.6	28.2	40.5	40.8	43.1	22.6	58.8	67.5
Total Wts	**598.3**	**387.0**	**589.8**	**654.5**	**708.5**	**537.1**	**654.4**	**658.3**