

## Quantifying the value of refuges for resistance management of transgenic Bt cotton

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### Introduction

The imminent release of transgenic cotton brings with it the need for some changes in production techniques to provide effective refuges as part of the pre-emptive resistance management strategy (see papers by Fitt and Forrester, this proceedings). Refuges are clearly the most unusual aspect of the strategy. Here we briefly describe our work to quantify the value of different types of refuge for *Helicoverpa*, where the chief measure of value is the number of moths produced per unit area over time. A refuge which produces twice as many moths is twice as valuable for resistance management, all else being equal.

### Methods

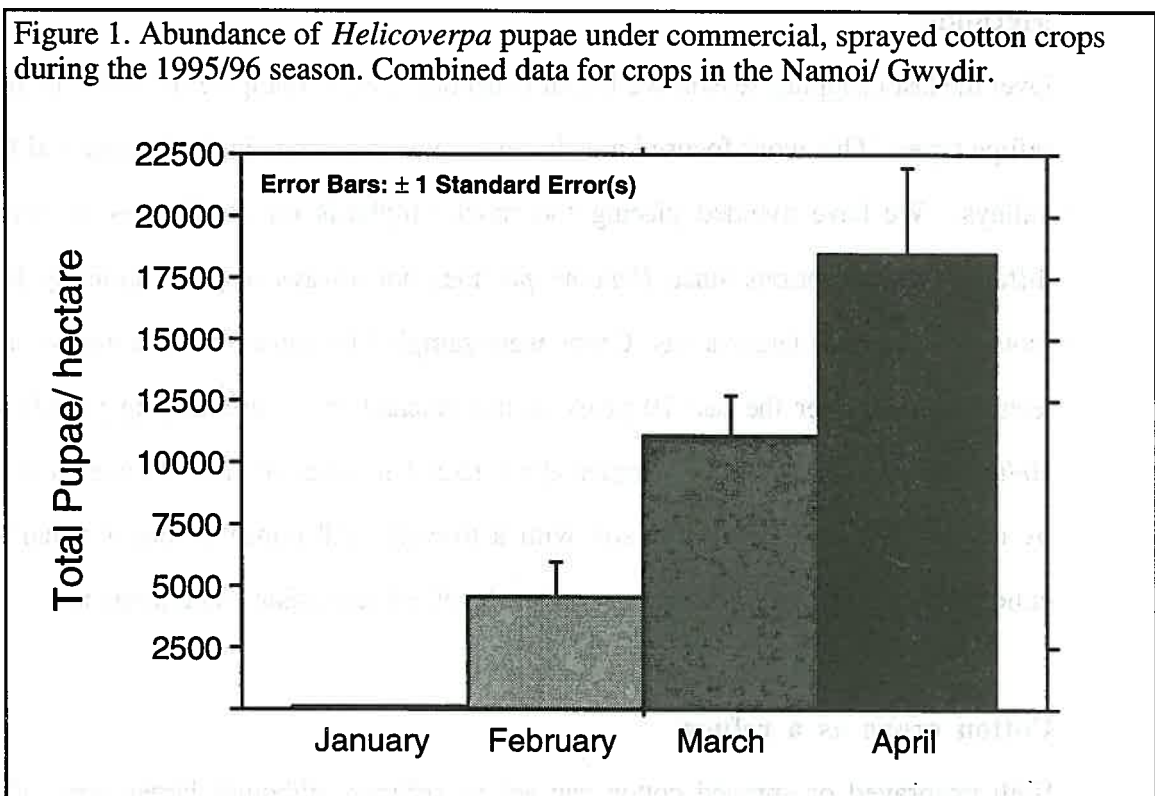
Over the last cropping season we began collecting data to compare the value of different refuge types. This work focused mostly on commercial crops in the Namoi and Gwydir valleys. We have avoided placing too much emphasis on small plots in comparing different refuge options since *Helicoverpa* does not always respond similarly to small plots as it might to larger areas. Crops were sampled for pupae using a well established technique used over the last 10 years in our research on overwintering populations of *Helicoverpa*. At each site we sampled about 15 x 1m<sup>2</sup> areas of soil. Pupae were located by carefully scraping away the soil with a trowel. All pupae or pupal remains were collected to provide information on species, levels of parasitism and emergence.

### Cotton crops as a refuge

Both unsprayed or sprayed cotton can act as refuges, although larger areas (5 times larger) are required with sprayed cotton. Until this year we had never sampled pupae in commercial cotton crops during the summer. We sampled a total of 24 crops each month

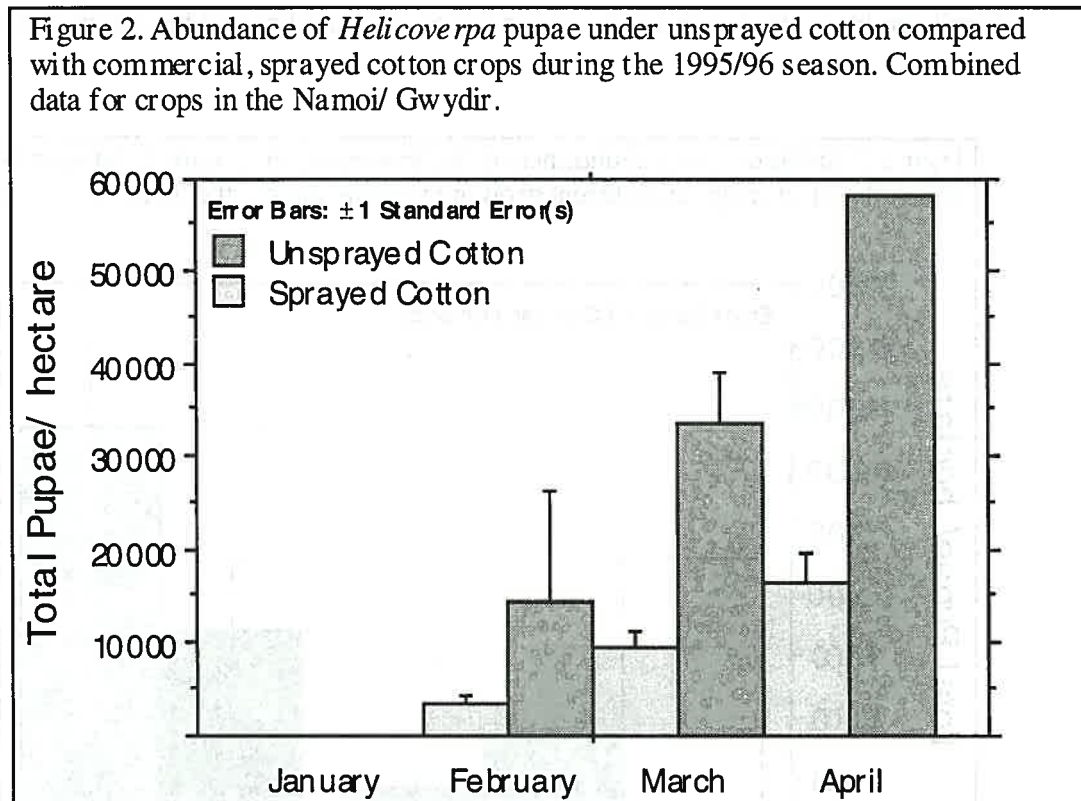
from early January onwards. These were located in the upper, central and lower Namoi and in the Gwydir (6 crops in each), some sampling was also done in commercial crops in the McIntyre valley during March 1996. We also sampled large areas of unsprayed cotton at six locations. These were areas of at least two hectares on research stations or associated with the unsprayed areas of INGARD cotton used for environmental impact studies.

Figure 1 shows the density of pupae averaged over all 24 commercial cotton fields each month. While very few pupae were present in January, numbers increased steadily through February, March and April reaching averages of almost 18,000 pupae/hectare by April. This is under sprayed cotton fields! These numbers reflect not only the high *H. armigera* pressure at that time of season, but also the increasing difficulties growers have in controlling this species with pesticides.



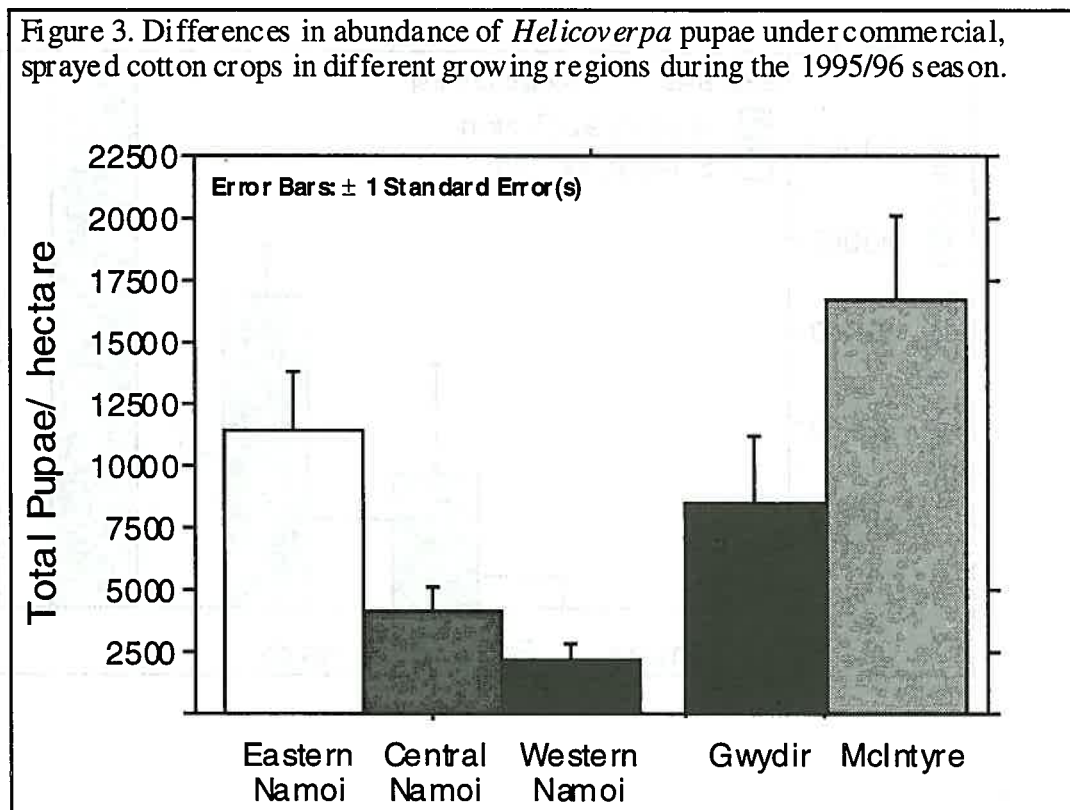
Not surprisingly, the unsprayed cotton crops averaged 4-5 times as many pupae as sprayed crops each month. (Figure 2). However, these are preliminary results only. It

is quite likely that we will find parasitism levels are higher in the unsprayed cotton with the result that numbers of moths actually produced from unsprayed crops may be only 3 times more than sprayed cotton. Overall these data support the 5 fold difference in areas of sprayed and unsprayed cotton required as refuges for next season (see Fitt, this proceedings).



There were also differences in the numbers of pupae/ha under sprayed crops in different regions. Crops in the eastern Namoi (Boggabri) averaged 5 times more pupae than those in the western Namoi (west of Wee Waa) (Figure 3). While we did not sample regularly in the McIntyre valley, our samples in March showed average densities under sprayed cotton of over 15,000/ha (1.5 pupae/m of row). While these crops were not being used as refuges for INGARD crops this season, these differences in pupal numbers between regions would not be a concern from the point of view of resistance management. The same differences between regions would have been seen with any refuge option and

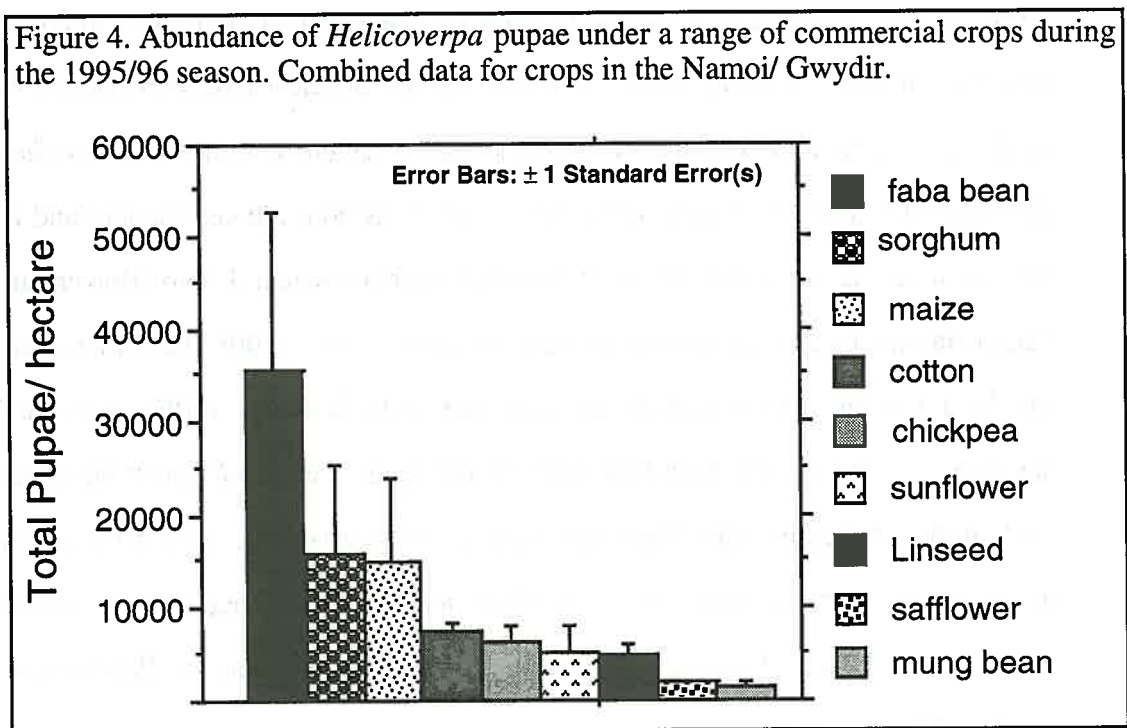
simply reflect differences in abundance of *Helicoverpa*. Sprayed cotton at Merah North is not a less valuable refuge than sprayed cotton at Boggabri, because the INGARD cotton at Merah North would also have had lower pressure from *H. armigera*. At Boggabri there would have been higher densities of *Helicoverpa* on an INGARD crop, but then the refuges would also be more productive. Provided the relative areas of refuge and INGARD crop are in place (as required by the strategy), then differences in *Helicoverpa* abundance from one region to another or one year to another will be accommodated.



### Other Crops as Refuges

All the other crop hosts of *H. armigera* where Bt sprays are not used will act as refuges. We studied many other commercial crops as they became available during the season. Data analysis is far from complete and so this is a preliminary picture only. Faba bean produced most pupae (Figure 4), but as a spring flowering crop many of these were *H. punctigera*. Summer flowering crops are more valuable since they produce moths when cotton is attractive to the moths and when transgenic cotton might produce resistant

moths. Sorghum and maize produced many pupae (all of which are *H. armigera*), twice as many per hectare as sprayed cotton crops. Specific refuges of unsprayed irrigated sorghum and corn (refuge option 3) can thus produce many moths but only over a period of two to three weeks. For that reason these refuges need to be managed to flower over a much longer period to be as effective as a cotton refuge. To achieve that will require multiple plantings of these crops.



None of the other crops studied (chickpea, sunflower, linseed, safflower and mung bean) produced as many pupae as cotton. In addition, chickpea, linseed, safflower are spring crops which will always produce a high proportion of *H. punctigera* and so have relatively less value for *H. armigera* resistance management unless they were grown extensively.

Small plot studies also showed the potential value as refuges of the grain legumes; pigeon pea and adzuki bean. December plantings which flowered during February produced the equivalent of 80,000-180,000 pupae/ha. These values are some 2-6 times higher than unsprayed cotton at the same time, suggesting that much smaller areas of these crops

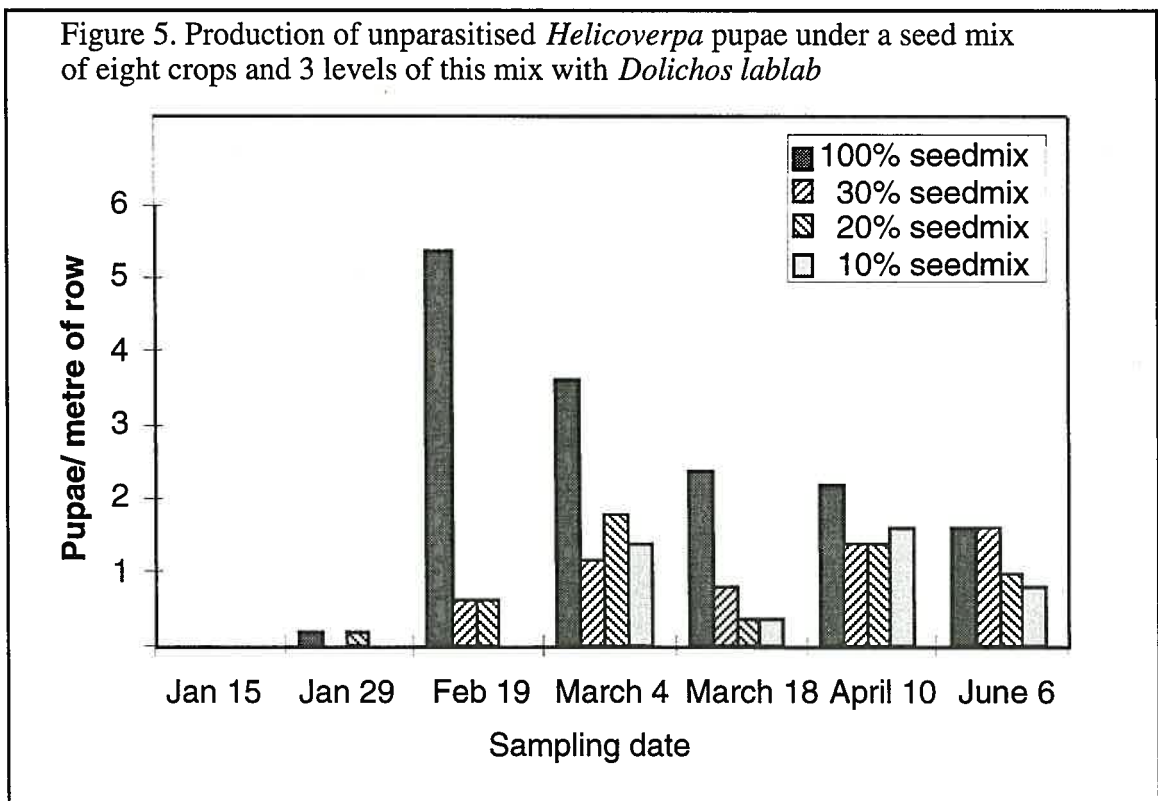
could be just as effective as refuges. In addition pigeon pea has the advantage of a longer flowering period (up to 5 weeks) than sorghum and corn. These options need much further research next season.

### **The Jungle Option.**

Another unusual refuge option being researched at Narrabri involves the use of a seed mixture of a range of *Helicoverpa* host plants. The mixture includes equal parts of eight crops: cotton, sorghum, corn, sunflower, pigeon pea, soybean, mungbean and nigerseed, all of which are hosts for *H. armigera*, but flower and develop at different times. We have used the seed mix in combination with the green manure legume, *Dolichos lablab*, to determine whether it is possible to use the *Dolichos* as both a green manure and a refuge for resistance management. We used *Dolichos* varieties which did not flower until very late in autumn. One treatment was the seed mix sown alone (100% seed mix), the others involved varying proportions of the seed mix with *Dolichos* (30%, 20% and 10% seedmix). So in the 10% seed mix, 90% of the seeds were *Dolichos* with about 1.3% each of the other eight crops. The experiment involved two replication of the 4 treatments all sown at the same time (late November) and monitored regularly to record plant establishment, plant phenology (flowering etc) and the numbers of *Helicoverpa* pupae produced during the season.

Results in Figure 5 shows two things. Firstly the 100% seed mix and the combinations with *Dolichos* did produce *Helicoverpa* pupae (almost all *H. armigera*), an average of 1.5-3.0 pupae/m at each sample date. Overall, the 100% seed mix produced about 3 times as many pupae as the 30%, 20% or 10% mix. These numbers of pupae are similar to numbers under the unsprayed cotton in the same field (2.1/m in February and 2.8/ m in March). Secondly and importantly, pupae were produced in the treatments over at least 3-4 months from the single sowing. All the crops were able to establish, reach flowering and mature when sown with *Dolichos*, although eventually it did smother most of the other plants and produced a large quantity of biomass. Agronomic implications of these

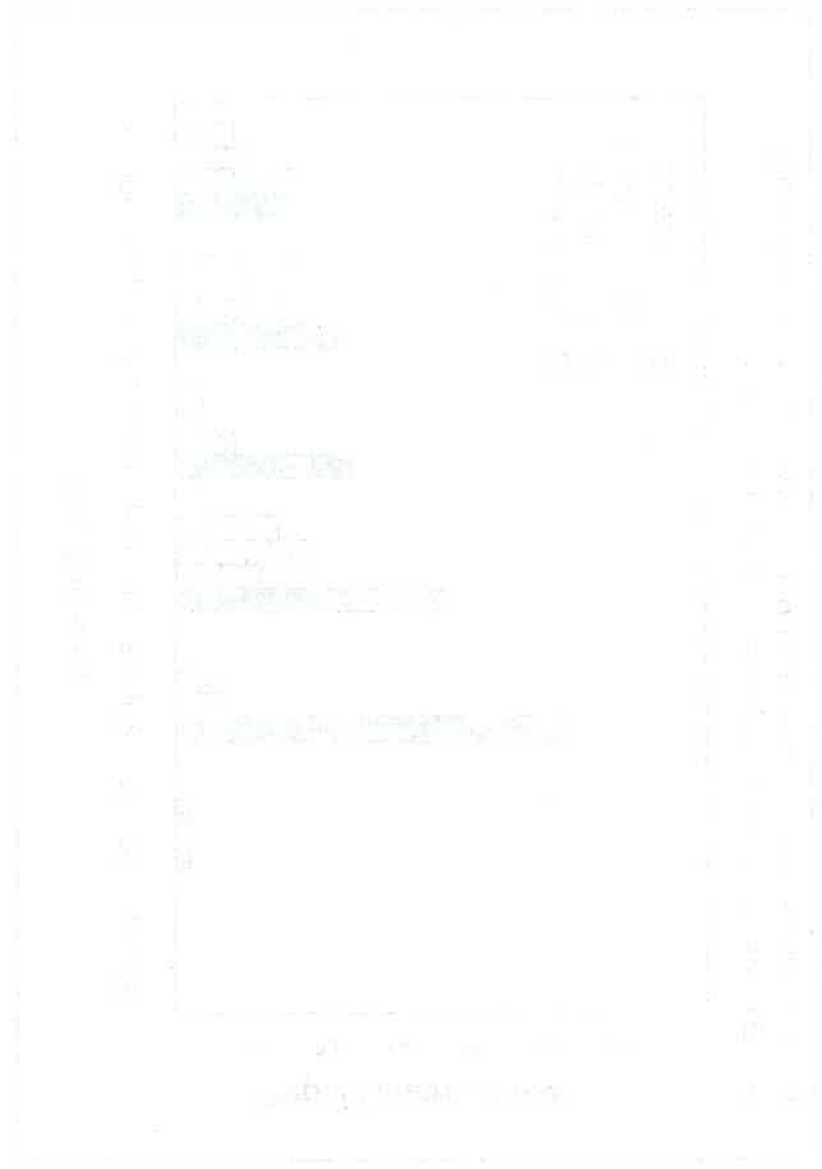
seed mix approaches are yet to be fully evaluated. For example, the seed mix plus *Dolichos* would return considerable organic matter to the soil, but perhaps not as much nitrogen as a pure *Dolichos* field. The fallen seeds from all the crops will also create a weed problem in the following crop so additional herbicide may be required. Nevertheless this demonstrates another possible refuge option which might impose minimal cost on growers since the green manure crops does not generate a harvest anyway. Clearly further work is needed to fully evaluate this and other novel refuge types. The main point is that there are many ways to implement effective refuges. We should not be blinkered into accepting the easiest option (sprayed cotton) in the long term.



### Conclusions

The results shown here must be viewed as preliminary and have been presented to outline gross patterns only. Much further analysis and research is required on these refuge options. Next season a variety of commercial refuges will be grown in association with research plots of INGARD cotton and monitored through the season to quantify their value.

The first part of the paper discusses the importance of the study and the objectives of the research. It highlights the need for a comprehensive understanding of the factors influencing the performance of the system under investigation. The study aims to identify the key variables and their interactions, which will help in optimizing the system's performance.



The second part of the paper presents the methodology used in the study. It details the experimental setup, the data collection process, and the statistical analysis performed. The results of the analysis are discussed in the following section, where it is shown that the identified factors have a significant impact on the system's performance. The study concludes with a summary of the findings and suggestions for future research.