05/13

Chemical blocking of seed set in weed beet: Final Report

Table of Contents

Executive Summary

Weed beet is an increasing problem in the UK, and current methods to keep populations in check are laborious, expensive, and often ineffective. If the production of viable seeds could be blocked in current populations of weed beet, then over time the seed bank will be gradually depleted. Certain chemicals can be applied to plants to prevent successful fertilization of the plants and seed set. Based on recent research, γ-aminobutyric (GABA) appeared to be a good candidate, and this was tested in glasshouse experiments. Since the GABA experiments were not successful, further compounds thought to affect seed set were tested in subsequent experiments (see p. 3 for background information).

Work Objectives

1. Test the effective concentration of applied GABA to intact, flowering plants to determine that which will block seed set.
2. Test when (relative to first pollen shed) applications of GABA are most effective.
3. As Objective 1, but by applying minidroplets of GABA solutions directly onto flowers.
4. Test the effect of applied BABA as a foliar spray or as a soil drench on seed set.
5. Test the effect of AVG (ReTain) as a foliar spray on seed set.
6. Test the effect of systemic fungicides applied prior to and around flowering on seed set.

Key findings

- GABA at a range of concentrations and application times had no effect on seed set in two separate experiments. Test plants included two sugar beet genotypes (SF/HS and Roberta). GABA also had no effect when applied directly to individual flowers.
- BABA was effective as a soil drench, and in one of two experiments was slightly effective as a foliar spray.
- AVG (aminoethoxyvinylglycine, ReTain®) was not effective as a foliar spray in one experiment.
- The systemic triazole fungicide Opus (epoxiconazole) was very effective and PunchC (flusilazole) slightly effective when applied just prior to flowering in weed beet.

Conclusions and looking ahead

Although GABA is a key factor in the fertilization process, there is no evidence that addition of exogenous GABA can disrupt internal concentration gradients sufficiently to block seed formation. Therefore, further experiments with GABA are not warranted. Alternatively, a simple fungicide application that blocks seed production in weed beet could be very beneficial, and therefore is worth further testing for confirmation and optimisation. With label approval, Opus could easily be applied to weed beet infested fields prior to or around flowering.

Results suggests that BABA may be effective in blocking seed set in weed beet. Applications as a soil drench, though effective, would not be practicable on a field scale; however, results of one experiment suggest a foliar spray may be effective. These experiments should be repeated, and if successful, application protocols can be optimised.

Chemical blocking of seed set in weed beet: Final Report

Background and rationale

Weed beet is an increasing problem in the UK, with more than 70% of UK beet fields infested, and current methods to keep populations in check are laborious, expensive, and often ineffective (1). Neglected weed beet populations introduce so many seeds into the soil that even after a 5 year rotation the amount of viable seed is not sufficiently diminished.

In theory, if the production of viable seeds could be blocked in current populations of weed beet, then over time the seed bank will be gradually depleted. There are many chemicals that can be applied to plants to prevent successful fertilization of the plants and seed set. Many of these substances induce male sterility and pollen abortion. However, these chemicals would not be effective since pollen from untreated plants could move large distances and pollinate weed beet plants. A more targeted approach would be to chemically induce female sterility or to block fertilization once a plant is pollinated.

One critical step in fertilization is the guidance of the pollen tube which emanates from germinating pollen) through the style of the female flower (Fig. 1). This tube contains the sperm cell and it must find the ovary containing the egg. Recent discoveries show that a chemical gradient within the maternal style tissue guides the pollen tube as it grows. Mutant plants that disrupt this gradient by over-producing the chemical inhibit fertilization (2,3). This chemical is γ-aminobutyric acid, or GABA. GABA is a ubiquitous non-protein amino acid that is naturally produced by plants (it is also a minor impurity in sugar beet roots). The reasoning behind the experimentation was that external application of GABA, at the correct concentration and at the right time, could raise levels within the flowers to block seed set. A further attraction was that GABA is relatively cheap, and is one of the main ingredients of a growth regulator (Auxi-Gro) already labelled for use on sugar beet in the USA.

During the course of the project, other chemical candidates were tested. β-aminobutyric acid (BABA) is another non-protein amino acid and chemically similar to GABA. BABA applied at high concentrations can also inhibit seed set in *Arabidopsis* (4). BABA probably acts by stimulating the over-production of callose in ovules, which retards development; only fully developed ovules can attract pollen tubes. (Reports also suggest that BABA has beneficial functions in 'priming' or stimulating plant defences against pests, again via callose formation [4]). Therefore, BABA was also tried.

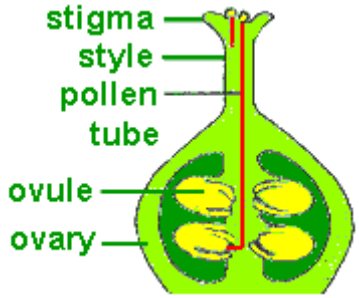

Fig. 1. *Schematic diagram of a typical flower. Seed set can be blocked by disrupting growth of the pollen tube, or by interfering with development of the ovule (the immature seed).*

The plant hormone ethylene is also required for proper ovule development, and if ethylene is blocked, excess callose is formed resulting in infertility (5). The compound

aminoethoxyvinylglycine (AVG) blocks ethylene action in plants, and is marketed as the product ReTain, a plant growth regulator labelled for use on apples and pears to prevent ethylene-induced premature fruit drop. The effect of ReTain on seed set was also tested.

There are anecdotal reports that fungicide use in the glasshouse reduced seed yield of plants (sugar beet and Arabidopsis) grown for producing seed for research purposes. Therefore, a range of systemic triazole and strobilurin fungicides were tested.

Experiments with GABA

Objectives

1) Test the effective concentration of applied GABA to intact, flowering plants to determine that which will block seed set.
2) Test when (relative to first pollen shed) applications of GABA are most effective.
3) As Objective 1, but using isolated branches fed with GABA solutions to increase and sustain GABA uptake.
4) As Objective 1, but by applying minidroplets of GABA solutions directly onto flowers.

Methods in brief

Two experiments were conducted on different genotypes. Experiment 1 tested GABA concentrations on flowering SF/HS plants (this is a KWS genotype which segregates for males sterility; these plants were fertile and shedding pollen). Plants were grown in May in a polytunnel greenhouse. Experiment 2 used Roberta, since hybrid bolters as well as weed beet introduce unwanted seed in beet fields. Plants were vernalised, then transferred to the glasshouse to allow bolting. When plants began to form flowers (prior to pollen shed; Fig. 5), plants were sorted into evenly distributed groups and randomly assigned a treatment. GABA solutions were prepared using Tween20 (polyoxyethelenesorbitan monolaurate) as a surfactant (0.05%, v/v). Tween or water alone was used as the control. Plants were sprayed until dripping with a hand-held pressurised sprayer. Plants were sprayed in order of increasing concentrations to avoid contamination in the sprayer. During applications, the treated plants were shielded with large screens to avoid drift onto neighbouring plants. In Experiment 2, applications were made during the entire course of pollen shed (approx. 2.5 weeks). Individual flowers were tagged with coloured wire and droplets of GABA were applied directly on the flower. Plants were harvested when seed balls turned brown and plants were beginning to die. Plants were bagged and dried for several more weeks. Seed yield was determined by hand threshing and sifting seed from trash, then by weighing all seed balls (<2 mm diameter) on each plant. Seed set was determined by selecting branches on similar positions of plants and counting the number of flowers on a 10 cm branch section. Percentage seed set was the ratio of the number of seed balls formed/number of flowers. Two branch sections per plant were measured. Data were analysed by ANOVA.

Key results

GABA had no effect on seed yield or seed set in any of the treatments (Fig. 2). GABA also did not affect the formation of seed balls when applied directly to flowers. The lack of effect could be due to rapid metabolism of exogenously applied GABA before it arrived in style tissues. Alternatively, it is possible that insufficient GABA penetrated the cuticle; however, studies using radiolabeled GABA indicated rapid uptake and subsequent breakdown (6). Objective 3 was omitted due to the lack of effect of foliar sprays.

Conclusions
GABA does not appear to be viable as a chemical treatment to block seed set. An alternative approach may be to use GABA analogues that have similar modes of action, but are metabolised at a much slower rate. However, the availability of these compounds may be limited, and health and environmental issues are unknown.

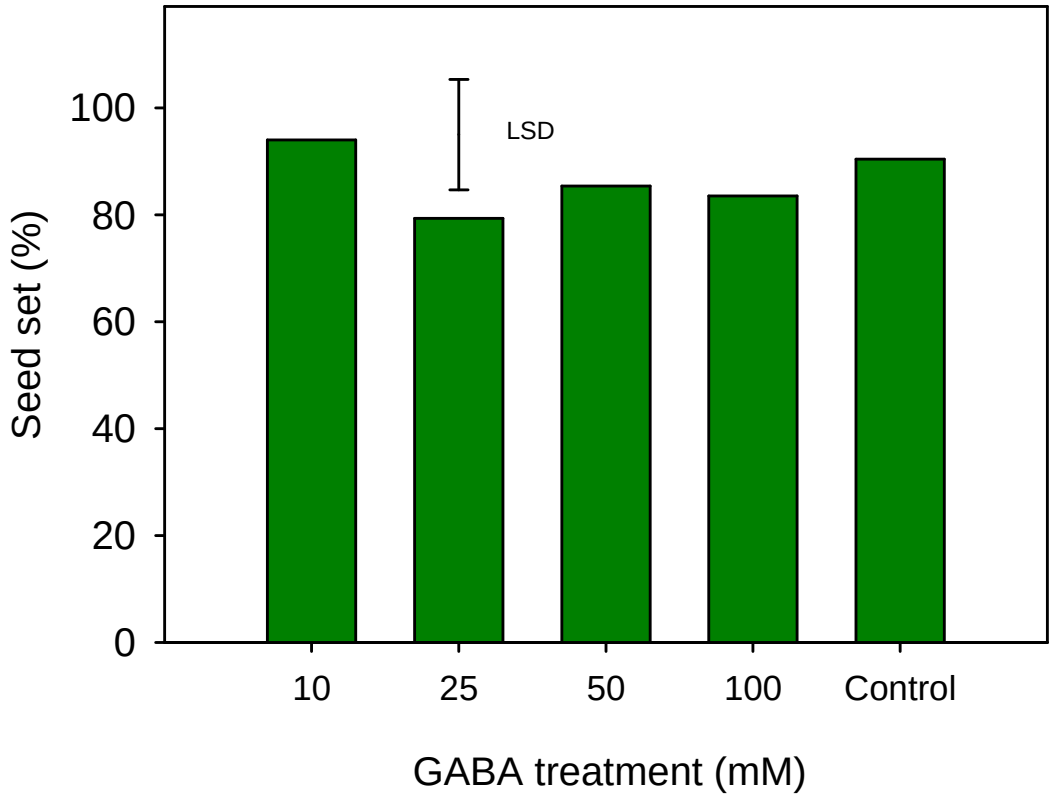

Fig. 2. *Effect of foliar spray applications of GABA on seed set on Roberta bolters in Experiment 2. Similar results were obtained in Experiment 1. The error bar represents the* $LSD_{0.05}$ *for treatment effect.*

Experiments with BABA and AVG

Objectives
- Test the effect of BABA applied at various concentrations on seed set.
- Compare BABA foliar spray vs. soil drench.
- Test the effect of AVG (ReTain) on seed set.

Methods in brief
Solutions of BABA were prepared and applied as in Experiment 2, above. A sample of ReTain, which is a wettable powder comprising 15% AVG, was obtained from East Malling Research. ReTain was applied at a rate of 0.8g L^{-1} according to the label. In these experiments, weed beet plants grown from locally collected seed were used. Following vernalisation, plants were grown and maintained in the growth chamber. Plants were divided

into 6 uniform groups and randomly assigned a treatment. The BABA soil drench was applied as 100 mL 1mM BABA onto the pot soil surface. A second application of treatments was given 7 days later. A 7th group of plants that bolted later than the first set used above was used to repeat the 1mM BABA spray treatment (BABA, 2nd experiment).

Key results

BABA applied as a foliar spray did not have any significant effect on seed set. The 10mM concentration seemed to improve seed set compared with controls (Fig. 3). However, when the 1mM BABA treatment was repeated on a second batch of plants from this group, seed set was significantly reduced. The different reactions in the two experiments is not clear, but may have been related to the age of the plants (although sprays were applied at the same stage of flowering). BABA applied as a soil drench was more effective than the sprays. In the *Arabidopsis* studies, BABA applied as a soil drench induced infertility (4,6). AVG did not have any noticeable effect on seed set.

Conclusions

The results from one experiment, or two experiments with conflicting results, are not sufficient to make a full judgment on the efficacy of the chemicals tested here. However, the data indicate that BABA may be effective in reducing seed set if applied in the correct fashion at the correct time. Clearly, soil drenches are not practical on a field scale, but field-scale spray application of BABA is possible (7), and further work may be warranted. On one experiment at one concentration, AVG did not appear effective. The experiments should be repeated to verify the results.

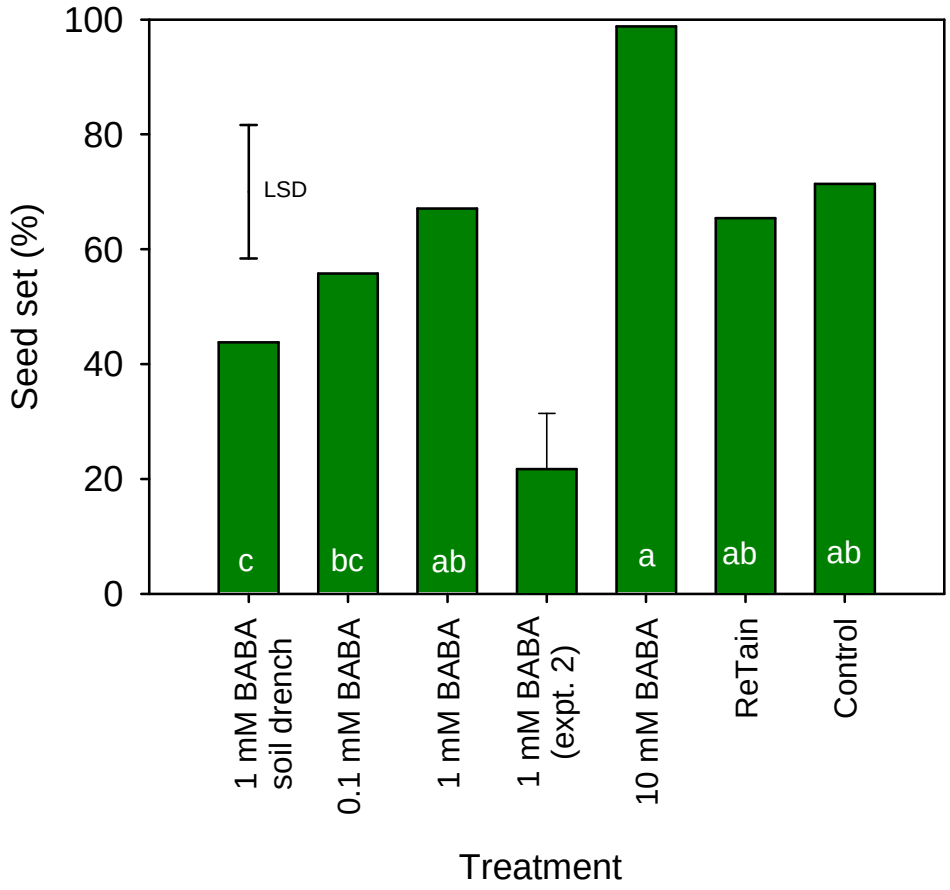

Fig. 3. *The effect of BABA and AVG (ReTain) on seed set on weed beet. Chemicals were applied as a foliar spray or as a soil drench, as indicated. Error bar represents the* $LSD_{0.05}$ *for the treatment effect in Experiment 1. Bars representing means denoted by the same letters are not significantly different using Fisher's LSD. Slightly greater seed set in the 10 mM BABA treatment may be due to experimental variation. Only the 1mM BABA treatment was tested again in a second experiment; error bar indicates the mean ± se (n = 6 plants).*

Experiments with systemic fungicides

Objective

- Test the effect of several common fungicides on seed set.

Methods in brief

Bolting Roberta plants were grown in the glasshouse and divided into five uniform groups and randomly assigned a treatment. Comet (pyraclostrobin), Opus (epoxiconazole), Cabaret (cyproconazole), PunchC (flusilazole plus carbendazim) were applied at labelled rates using a hand-held sprayer. Water was used as the control. Plants were treated once.

Key results

Opus had a significant and dramatic effect on seed set compared with controls (Fig.4). PunchC had a smaller, but still significant effect. Cabaret and Comet did not detectably alter seed set.

Conclusion

The results corroborate anecdotal reports that triazole fungicide use in glasshouses adversely affects seed production. It is not clear why Opus had a larger effect than other triazoles. The mechanism of action is unknown, although there is substantial evidence that systemic fungicides can affect sugar beet physiology independently of disease control activity. These results are encouraging, but need verification with further tests.

Future directions

It remains a viable possibility that chemical treatments can be used to block seed production in weed beet. The chemicals that show some promise (Opus, BABA) have potential to be inexpensive and easy to apply options that can be used in combination with other control methods. Additional experimentation is required in order to decide if these products merit further development for this use.

References

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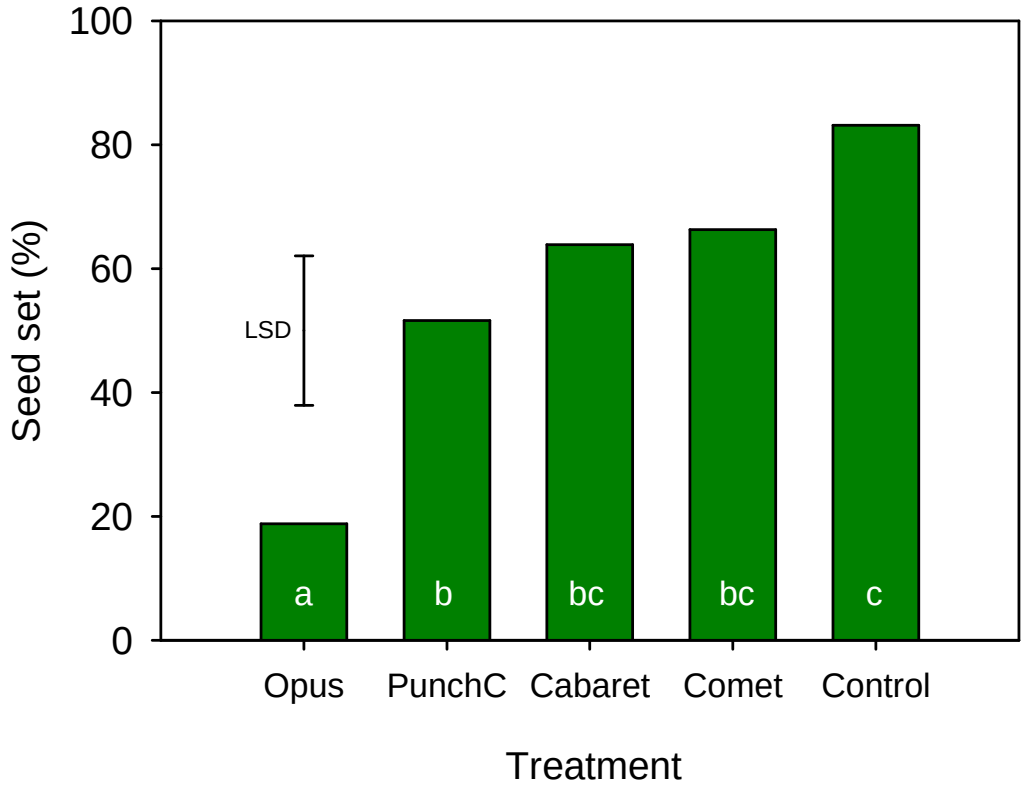

Fig. 4. The effect of systemic fungicides on seed set on weed beet. Error bar represents the $LSD_{0.05}$ for the treatment effect. Bars representing means denoted by the same letters are not significantly different using Fisher's LSD.

Fig. 5. Flowering branches at the time of treatment before flowers open (A), and through pollen shed (B).

Project résumé: Expenditure and scientific staff input for 2005

What was the planned expenditure?	£14,811
What was the actual expenditure?	£14,720.99
What was the planned scientific staff input in staff years?	0.3
What was the actual scientific staff input in staff years?	0.3
Have there been any outputs from the project this year?	NO
Have any opportunities for exploiting intellectual property been identified?	NO
Are there any scientific opportunities arising from this project that have not been mentioned previously?	NO

DECLARATION

I declare that the information I have given is correct to the best of my knowledge and belief.

Signature: Date: