Crop Response to Leaf and Seed Applications of the Biostimulant ComCat® under Stress Conditions

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Abstract: Although clear evidence for benefits in crop production is partly missing, several natural compounds and microorganisms have been introduced to the market as biostimulants. They are supposed to enhance nutrient efficiency and availability in the rhizosphere, reduce abiotic stress, and improve crop quality parameters. Biostimulants often derive from natural compounds, such as microorganisms, algae, and plant extracts. In this study, the commercial plant extract-based biostimulant ComCat® was tested in two field experiments with maize in the communities of Banikoara and Matéri in Northern Benin and six pot experiments (four with maize and two with winter barley) at the University of Hohenheim in Germany. Maize was grown under nutrient deficiency, drought, and weed competition, and winter barley was stressed by the herbicide Luximo (cinmethylin). ComCat® was applied at half, full, and double the recommended field rate (50, 100, and 200 g ha⁻¹) on the stressed and unstressed control plants as leaf or seed treatment. The experiments were conducted in randomized complete block designs with four replications. The above-ground biomass and yield data of one experiment in Benin were collected. The biostimulant did not promote maize and winter barley biomass production of the unstressed plants. When exposed to stress, ComCat® resulted only in one out of eight experiments in higher barley biomass compared to the stressed treatment without ComCat® application. There was a reduced phytotoxic effect of cinmethylin after seed treatment with ComCat®. Crop response to ComCat® was independent of the application rate. Basic and applied studies are needed to investigate the response of crops to biostimulants and their mechanisms of action in the plants before they should be used in practical farming.

Keywords: biocontrol; biofertilizers; natural compounds; plant extracts; plant growth stimulant

1. Introduction

Biotic and abiotic stresses account for 13–94% yield losses of agricultural crops worldwide. Through site-specific management practices and the selection of tolerant crop cultivars, farmers put high effort in reducing yield losses due to drought, salinity, unfavorable temperatures, weed competition, diseases, and pests [1–3]. Numerous natural organic and inorganic compounds and microorganisms have been described to promote plant growth (and yields) or induce defense mechanisms in plants against abiotic and biotic stresses [4]. Those natural compounds are defined as biostimulants. In contrast to synthetic pesticides and mineral fertilizers, biostimulants are promoted to provide an ecological friendly and easy-to-handle option to favor crop growth and suppress stresses. However, their effect
seems to be less evident and more difficult to quantify. Often, their mode of action has also not been discovered [4–6].

In Western Africa, the main focus of using biostimulants is to compensate for soil fertility decline, replacing synthetic fertilizers in crops production and reducing the process of soil degradation and environmental pollution. Therefore, the use of biostimulants may supply the use of synthetic fertilizers and perhaps can increase the nutrient availability in the soil [7]. The association of mung bean (*Vigna radiata* L.) Wilczek with maize (*Zea mays* L.) constitutes also a promising cultural practice in soil restoration and sustainable management on peripheral areas of the W-Arly-Pendjari Biosphere Reserve through biological nitrogen fixation by mung bean rhizobia.

One group of biostimulants originates from plant extracts containing allelochemicals [3]. Allelochemicals play an important role in the interaction between plant species and defense mechanisms. They are involved in plant stress reactions such as mechanical wounding, disease infection, competition with other plants, and insect attack. The release of allelochemicals occurs via the leaves and roots of the plants. However, the duration varies depending on plant species, plant growth stage, and environmental conditions [8,9].

ComCat® is a commercial biostimulant offered by the German company AgraFörUm. It is produced by extraction of the perennial plant (*Lychnis viscaria* L.) Borkh and the biostimulatory efficacy is based on the two brassinosteroids: 24-epi-secasterone and 24-epicastasterone [10–12]. The recommended field rate of the product is 100 g ha\(^{-1}\). Extracts were formulated as water-dispersible powder containing 25% (weight/weight) of active substance. The extract is supposed to promote the general health of mono- and dicotyledonous crop plants and stimulate defense mechanisms against abiotic and biotic stress. However, only one scientific paper underlines those benefits for vining peas (*Pisum sativum* L.) and mixtures of peas with spring field beans (*Vicia faba* L.) [13]. As with other plant hormones, there is no single molecular mode of action or target site known for brassinosteroids. They act as signaling compounds and can trigger various effects ranging from growth stimulation to stress protection depending on growing conditions [12]. Moreover, brassinolide (BL), the most bioactive form of the growth-promoting plant steroids, can protect plants from various environmental stresses as moderate heat, cold, drought, salinity, heavy metal, and herbicidal injury [13]. They also act as regulating agents involved in vitamin E production, which indirectly protects cell membranes against free radical damage, especially during drought stress, and they are also involved in crop growth and development, especially root development, via cell elongation and division [13].

The objective of this study was to analyze the crop response to ComCat® applications under different stress conditions. Stress was induced by drought, nutrient deficiency, weed competition, and herbicide application with reduced selectivity. It was hypothesized that early post-emergence applications of ComCat® increased maize biomass under drought, nutrient deficiency, and weed competition conditions. We further hypothesized that ComCat® can reduce herbicide stress in barley after seed and leaf treatment. It was also hypothesized that biomass of the crops responds positively to increasing rates of ComCat®.

2. Materials and Methods

2.1. Experimental Sites, Details, and Data Collection

From 2019 until 2021, one field experiment and three pot experiments were conducted to test the response of maize, mixed maize mung bean, and barley (*Hordeum vulgare* L.) to leaf and seed treatments of the biostimulant ComCat® under stress conditions. Each of the four experiments was replicated twice (experiment A and B) with the same protocols.

2.1.1. Field Experiments with Maize and Mixed Maize–Mung Bean in Benin to Investigate the Effect of ComCat® under Nutrient Deficiency

The experiments were carried out on peripheral areas of the agro-sylvo-pastoral W-Arly-Pendjari Complex in the communes of Matéri (10°45.727' N, 1°11.703' E and 227 m a.s.l (above sea level)) and Banikoara (11°14.48’ N, 2°39.4540’ E and 231 m a.s.l.) in
Northern Benin in 2020. The climate is tropical with one rainy season from May to October corresponding also to the growing season of maize and other annual crops. Annual rainfall in 2020 was 1150 mm. The soil type at both sites was ferruginous tropical loamy sand with a pH value of 6.5 and a carbon content of 0.7%.

It was tested if ComCat® can compensate for nutrient deficiency in maize. Plots had a size of 4 m width and 6 m length. Maize, cv. DMR-ESRW, was sown at a density of 6.25 plants m\(^{-2}\). The experimental design was a randomized complete block with four repetitions. Treatment 1 was the control of maize without ComCat® and fertilizer. Treatment 2 was an intercropping of maize and mung bean, cv. Wilczek (12.45 plants m\(^{-2}\)) representing an organic N-fertilizer. In treatments 3–5, maize with 50, 100, and 200 g ha\(^{-1}\) ComCat® as post-emergence application with a knapsack sprayer (200 l water ha\(^{-1}\)) at the 2-leaf stage (10 DAS) was tested. In treatment 6, maize received mineral fertilization (59 kg N ha\(^{-1}\), 17 kg P\(_2\)O\(_5\) ha\(^{-1}\), 17 kg K\(_2\)O ha\(^{-1}\), 5 kg SO\(_4\) ha\(^{-1}\), 0.5 kg B ha\(^{-1}\), 1 kg Zn ha\(^{-1}\)) and no ComCat®. Manual weeding was done 15 days after sowing (DAS) at both sites and again 45 DAS at Banikoara. Maize fresh biomass was sampled and weighted 28 DAS at both locations and 42 and 56 DAS at Banikoara using a square frame of 0.5 m\(^2\) in each plot. Maize grains were harvested by hand and weighted for each plot 84 DAS.

2.1.2. Pot Experiments with Maize in Germany to Investigate the Effect of ComCat® under Drought Conditions

The experiments were set up in the greenhouse of the University of Hohenheim (48°72′ N, 9°12′ E) in 2020 (exp. A and B) as a randomized complete block design with water content in the pot (80% and 40% water-holding capacity) as the first factor (Figure 1) and application of ComCat® as the second factor. Each treatment was repeated four times. Maize, cv. Danubio (DSV-Saaten, Isernhagen, Germany), seedlings were transplanted into 18 cm × 18 cm × 17 cm plastic pots (Kakteen-Haage, Erfurt, Germany) filled with a substrate of 50% compost, 25% loam, and 25% sand. Each pot contained two maize plants. ComCat® was applied at 2 leaf stage of maize (18 DAS) with a precision application chamber using a Teejet nozzle (8002 EVS, Teejet® Spraying Systems Co., Wheaton, IL, USA). The application chamber was calibrated for a volume of 200 L ha\(^{-1}\), at a speed of 800 mm s\(^{-1}\), a distance of the spray nozzle of 500 mm above the sprayed surface and a spraying pressure of 300 kPa. Four rates of ComCat® were tested (0, 50, 100 (recommended field rate), and 200 g ha\(^{-1}\)). Pots were fertilized once at 24 DAS with 2 g NPK(MgO)-fertilizer (14 + 7 + 17(+2)), Compo Blaukorn NovaTec®, Münster, Germany. Plants were grown at 20 °C during the day and 15 °C at night with 12 h/12 h assimilation lightning.

Figure 1. Untreated maize plants before harvest (47 DAS) with sufficient irrigation water (left) and reduced irrigation water (right).
Maize shoots were harvested 47 DAS and dried for 48 h at 80 °C. Then, the dry weight of the above-ground biomass was determined.

2.1.3. Pot Experiments with Maize in Germany to Investigate the Effect of ComCat® under Weed Competition

Experimental details were equal to 2.1.2 except for exposing maize to weed competition instead of drought. Five seedings of four weed species (*Alopecurus myosuroides* Huds., *Echinochloa crus-galli* L., *Abutilon theophrasti* L., *Solanum nigrum* L.) commonly occurring in maize were transplanted in the cotyledon stage two days after sowing of maize to simulate early weed competition (Figure 2). ComCat® was applied at the 2-leaf stage of maize (17 DAS).

The weed and maize shoot biomass was harvested 43 DAS and then dried for 48 h at 80 °C. Then, the dry weight of the above-ground biomass was determined.

2.1.4. Pot Experiment with Winter Barley in Germany to Investigate the Effect of ComCat® under Herbicide Stress

The pot experiments were set up as multifactorial, randomized complete blocks with four repetitions of each treatment at the University of Hohenheim in 2019 (exp. A) and 2020 (exp. B). The first factor was the application of the pre-emergence herbicide Luximo (a.i. cinmethylin) at the rates of 0 and 0.66 L ha$^{-1}$. The second factor was the application of ComCat® at rates of 0, 50, 100, and 200 g ha$^{-1}$. The third factor was the type of application with an early post-emergence application co-formulated with Luximo and a seed treatment of ComCat followed by an early post-emergence application of the herbicide 7 DAS with a precision application chamber using a Teejet nozzle (8002 EVS, Teejet® Spraying Systems Co., Wheaton, IL, USA). The application chamber was calibrated for a volume of 200 L ha$^{-1}$, at a speed of 800 mm s$^{-1}$, a distance of the spray nozzle of 500 mm above the sprayed surface and a spraying pressure of 300 kPa. The seed treatment was conducted with 1 g L$^{-1}$ Luximo, and 10, 20, and 50 mg kg$^{-1}$ of ComCat® dissolved in water and formulation ingredients (sticker, dye). Then, seeds were placed into this solution for 30 s. Winter barley, cv. Sensation (DSV-Saat, Isernhagen, Germany), seedlings were transplanted into 8 cm
In each pot, four barely seeds were placed 3 cm deep in the soil. Then, plants developed under a 20 °C/15 °C day/night temperature and 12 h/12 h light/dark regime. Barley shoots were harvested 21 and 27 DAS and dried for 48 h at 80 °C before the above-ground dry biomass was determined.

2.2. Data Analysis

The data were analyzed with the R Studio software (Version 1.0.136, RStudio Team, Boston, MA, USA). Prior to the analysis, the data were tested for homogeneity of variance and normal distribution of the residues. A (multifactorial) analysis of variance (ANOVA) was performed, and the means of every observation were compared with Tukey’s HSD test at α ≤ 0.05. The following model was used for one-factorial analysis of variance.

\[ Y_{ijk} = \mu + a_i + b_k + e_{ik} \]  

where \( Y_{ijk} \) is the result (e.g., grain yield) of treatment \( i \) in block \( k \); \( \mu \) is the general mean, \( a_i \) is the yield attributed to treatment \( i \), while \( b_k \) is the block effect of block \( k \), while \( e_{ik} \) is the residual error of that specific plot. Multifactorial ANOVA was done accordingly.

3. Results

3.1. Field Experiments with Maize and Mixed Maize–Mung Bean in Benin to Investigate the Effect of ComCat® under Nutrient Deficiency

None of the treatments at Matéri had a significant effect on maize biomass until 28 DAS (Figure 3). Then, the experiment was flooded after heavy rainfall. Further biomass cuts and harvest were not possible. At Banikoara, synthetic fertilizers significantly increased the average grain yield to 2.1 t ha\(^{-1}\) (±1.01), which is more than 50% higher compared to the control plots and the maize–mung bean intercropping. The ComCat applications did not generate higher yields in comparison to the control (Figure 4).

![Figure 3](image-url)  

**Figure 3.** Maize dry biomass in response to maize–mung bean intercropping (MB+M), ComCat® (CC) treatments at rates of 50, 100, and 200 g ha\(^{-1}\) and mineral fertilizer application (NPK) at Matéri, Benin, 28 DAS. Treatments with identical letters above columns did not differ significantly based on Tukey’s HSD test.
Figure 3. Maize dry biomass in response to maize–mung bean intercropping (MB + M), ComCat® (CC) treatments at rates of 50, 100, and 200 g ha\(^{-1}\) and mineral fertilizer application (NPK) at Maté ri, Benin, 28 DAS. Treatments with identical letters above columns did not differ significantly based on Tukey’s HSD test.

Figure 4. Maize grain yield in response to maize–mung bean intercropping (MB + M), ComCat® (CC) treatments at rates of 50, 100, and 200 g ha\(^{-1}\) and mineral fertilizer application (NPK) at Banikoara, Benin, 84 DAS. Means with identical letters above columns do not differ significantly based on Tukey’s HSD test ($p \leq 0.05$).

3.2. Pot Experiment with Maize in Germany to Investigate the Effect of ComCat® under Drought Conditions

Drought significantly reduced maize dry biomass in both experiments. However, the effect was stronger in experiment A than in experiment B. Maize biomass production was higher in experiment A than in experiment B, although experimental conditions were similar. In both experiments, the dry biomass of maize with sufficient water supply was slightly increased by ComCat®. However, treatments were not significantly different. The application of ComCat® did not affect the biomass of maize, especially under drought conditions (Figure 5).

Figure 5. Cont.
slightly increased by ComCat®. However, treatments were not significantly different. The application of ComCat® did not affect the biomass of maize, especially under drought conditions (Figure 5).

Figure 5. Maize biomass (g pot$^{-1}$) in response to drought stress and ComCat® application at 50, 100, and 200 g ha$^{-1}$ at Hohenheim 2020, 47 days after sowing in the experiments (A, B). A rate of 100 g ha$^{-1}$ corresponds to the recommended field rate. Wet = 80% field capacity, Dry = 40% field capacity. Means with identical letters above the columns do not differ significantly based on Tukey’s HSD test ($p \leq 0.05$).

3.3. Pot Experiment with Maize in Germany to Investigate the Effect of ComCat® under Weed Competition

Weed competition and ComCat® treatments did not affect maize dry biomass in experiment 6A (Figure 6). However, weed competition caused an approximately 70% biomass decline in experiment 6B. This might be explained by the higher weed biomass in experiment B (Figure 6). In experiment A, weed dry biomass was recorded at 7–9 g per pot, whereas in experiment B, more than 25 g of weed dry biomass was measured. Evidently, in experiment B, limited resources available for biomass production were exploited by weeds to a greater extent than in experiment A. ComCat® did not affect maize and weed dry biomass (Figures 6 and 7) in both experiments.

Figure 6. Cont.
pot, whereas in experiment B, more than 25 g of weed dry biomass was measured. Evidently, in experiment B, limited resources available for biomass production were exploited by weeds to a greater extent than in experiment A. ComCat® did not affect maize and weed dry biomass (Figures 6 and 7) in both experiments.

Figure 6. Maize biomass (g pot\(^{-1}\)) in response to weed competition and ComCat\(^{\circledR}\) application at 50, 100, and 200 g ha\(^{-1}\) at Hohenheim 2020, 43 days after sowing in the experiments (A, B). A rate of 100 g ha\(^{-1}\) corresponds to the recommended field rate. C = weed-free control, Weed = 20 plants per pot transplanted at time of maize emergence. Means with the same letters above the columns do not differ significantly based on Tukey’s HSD test (\(p \leq 0.05\)).

Figure 7. Weed biomass (g pot\(^{-1}\)) at Hohenheim 2020 in experiments (A, B), 43 days after sowing and application of ComCat\(^{\circledR}\) at 50, 100, and 200 g ha\(^{-1}\) in the experiments (A, B). A rate of 100 g ha\(^{-1}\) corresponds to the recommended field rate. C = weed-free control, Weed = 20 plants per pot transplanted at time of maize emergence. Means with the same letters above the columns do not differ significantly based on Tukey’s HSD test (\(p \leq 0.05\)).
3.4. Pot Experiment with Winter Barley in Germany to Investigate the Effect of ComCat® under Herbicide Stress

Luximo caused a significant biomass loss in winter barley. This became more obvious in experiment A than in experiment B. Seed treatment with ComCat® and Luximo could reduce herbicide stress in experiment A independent of concentration. The winter barley dry biomass of early post-emergence application of co-formulated ComCat® and Luximo were equal to the treatment with solely Luximo, indicating that herbicide stress was only reduced by seed treatment with ComCat®. Without herbicide stress, ComCat® did not affect winter barley dry biomass (Figure 8).

![Graph showing winter barley biomass response to post-emergence application of Luximo (Lux) and ComCat (CC) as seed (S) and co-formulated post-emergence (P) applications at 50, 100, and 200 g ha\(^{-1}\) at Hohenheim 2021, 21 and 27 days after sowing in the experiments (A,B). A rate of 100 g ha\(^{-1}\) corresponds to the recommended field rate. Means with identical letters above the columns graph do not differ significantly based on Tukey’s HSD test (\(p \leq 0.05\)).](image)

4. Discussion

The hypothesis that early post-emergence applications of ComCat® increased maize biomass under drought, nutrient deficiency, and weed competition in these experimental conditions has to be rejected. The biostimulant ComCat® did also not promote maize and winter barely biomass production under favorable growing conditions regardless if it was applied at low or high rates. These results contradict to studies in New Zealand, where ComCat® increased vining pea, pea, and spring bean yields by 2, 11, and 15% compared to the conventionally treated crops without ComCat® [13]. The physiological reactions to ComCat® application was not investigated in the present research. In the New Zealand
study, it was reported that the brassinosteroids (BRs) contained in ComCat® protected plants from various environmental stresses, such as moderate heat, cold, drought, salinity, heavy metal, and herbicidal injury. The authors also found that BRs acted as regulating agents involved in vitamin E production that indirectly protected cell membranes against free radical damage, especially during drought stress, and they are also involved in crop growth and development, especially root development, via cell elongation and division [13].

Defense reactions against abiotic and biotic stresses induced by biostimulants were explained by stimulation of soil microbial activity, root formation, and plant metabolism [14–18]. If those effects of ComCat® would have occurred in the present study, it should have resulted in higher crop biomass.

In one experiment of the present study, ComCat® as seed treatment reduced the phytotoxic herbicide effects in barley compared to standard herbicide treatments without ComCat®. That effect could be explained by a faster degradation of cinmethylin in barley when ComCat® was applied on the seeds. It might be the case that ComCat® induced the activity of certain enzymes in barley, which are involved in herbicide metabolism. The higher activity of herbicide metabolizing enzymes such as glutathione-S-transferase and P450-monoxygenase has often been described as the reason for herbicide selectivity and herbicide resistance in tolerant plants [19]. The mode of action of ComCat® in plant defense mechanisms has not been explored. Therefore, it is not clear if ComCat® can stimulated the herbicide metabolism. In general, the mechanisms induced by biostimulants in crops are often not understood. Modern analytics including omic technologies have been applied to understand biostimulants activity [6]. That would help to explain why biostimulants in some cases promoted crop growth under drought or salinity and improved the nutrient use efficiency of crops [6]. It could also facilitate selecting the right biostimulants at the right dose for different crops and under different growing conditions to strengthen crop development.

Although there was no significant difference between maize grain yields obtained with the maize single cropping system and its intercropping with mung bean, the latter allowed a double crops harvest during the same cropping season. Similar results were observed under a maize–mung bean intercropping system on a tropical ferruginous soil in western Burkina Faso [20] and under a millet–mung bean intercropping system in Senegal [21]. In Senegal, millet yield increased under a millet–mung bean intercropping at a 1:1 ratio [22]. The intercropping ratio of 1:1 (millet/mung bean) was more productive compared to the other mixtures. This cropping system promotes atmospheric nitrogen fixation, and the produced biomass contributes to soil structure and organic matter content improvement. In addition, legumes are recognized as an atmospheric nitrogen fixator that recycles it in the cropping systems [23], allowing fertilizer application rate reduction. This cropping system represents a promising strategy for sustainable soil fertility management [20] and food security in a context of climate change in West Africa. However, ComCat® did not show any effect as biofertilizer. It is also questionable if plant extracts have such effects, since biofertilizers usually derive from living microbes, increasing the availability of nutrients in the rhizosphere [24].

There is sufficient evidence that biostimulants can promote crop growth and reduce stress symptoms under water and nutrient deficit, salinity, and unfavorable temperatures. However, it seems that these effects depend on several factors including the timing and type of application, crop species, and growth stage and stress intensity [6]. In the present study, ComCat® was applied with and after crops had been exposed to stress. Bulgari et al. [6] point out in their review that biostimulants might have to be applied before the stress occurs. If biostimulants could be provided with a detailed label describing the proper timing and rate of application and the mode of action in different crops, their practical use could be improved and the waste of product could be avoided [6].
5. Conclusions

None of the hypotheses have been demonstrated. In the present studies, ComCat® did not fulfill the expectations of a biostimulant according to the EU-regulation Nr. 2019-1009, saying that biostimulants should provide better nutrient efficiency or abiotic stress tolerance. There was just one positive effect of ComCat when applied on barley seeds before an herbicide stress induction, but it could not be replicated. It might be true that ComCat® induced less evident physiological processes such as the activation or de novo synthesis of enzymes as described. However, those physiological processes have not been investigated in this study. It can also be concluded that ComCat® did not promote crop biomass production and yield both under favorable and high stress conditions.

Author Contributions: All authors contributed extensively to this work. A.H., M.M., H.O. and F.N.O. organized the setup of the experiments and collected the data. A.A., V.A.P.A., B.L.S.D., A.K.D.A., F.N.O., M.M. and R.G. analyzed the data, evaluated the results and drafted the manuscript. H.-J.S., H.O. and F.N.O. helped in the concept of the study, designing the experiment, the drafting and revision of the paper. All authors have read and agreed to the published version of the manuscript.

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