Development of CO_2 releasing beads as attractive component for novel « attract-and-kill » formulations to protect crops from soil-borne insect pests

Anant Patel[1*], Wilhelm Beitzen-Heineke[2], Marina Vemmer[1]
Univ Appl Sc, Eng & Alternative Fuels, Bielefeld, Germany (anant.patel@fh-bielefeld.de)

INTRODUCTION AND OBJECTIVE

As larvae of many herbivorous insects (e.g. wireworms, western corn rootworm and black vine weevil) use CO_2 for host location the use of attractants based on CO_2 is a promising approach for attract-and-kill strategies in pest control (Fig. 1). By attracting the larvae directly to the insecticide, conventional insecticide applications or other control strategies can be replaced, the amount of insecticides can be minimized and the environment and health of farmers and consumers can be protected.

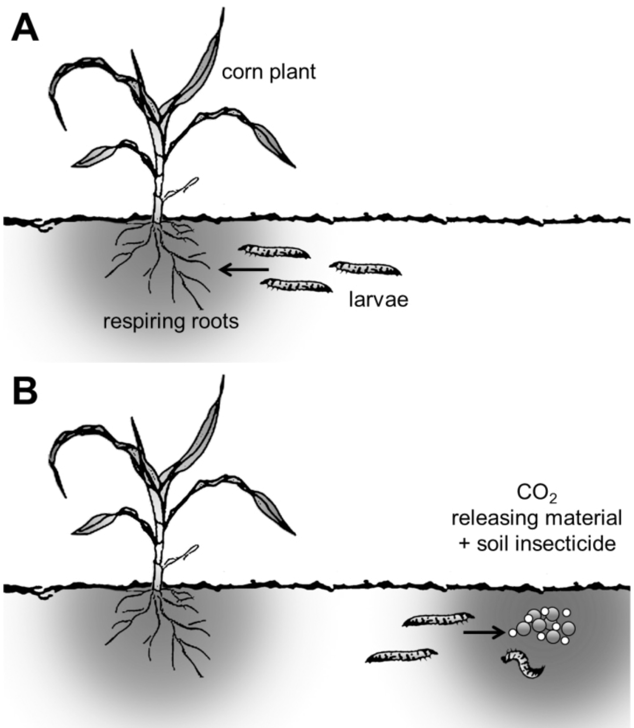


Figure 1: Larvae use CO_2 to locate the roots of living plants like corn, potato or strawberry (A). CO_2 releasing materials can attract the larvae in order to lure them away from the roots and have the potential to increase the efficacy of a soil insecticide within the scope of an attract-and-kill strategy (B).

This work focuses on the development of long-time CO_2 releasing beads as attractive component for "attract-and-kill"-formulations.
For the construction of a CO_2 release system baker's yeast has proved to be successful (Vemmer 2011). Encapsulation methods can improve the protection of the cells in soil, the release of CO_2 and the handling of the resulting product.

MATERIALS AND METHODS

Encapsulation of Saccharomyces cerevisiae
As CO_2 source, a commercial baker's yeast mixture or a pure *S. cerevisiae* culture was encapsulated in moist Ca-alginate beads without and with additives. A negative control was composed of the same formulation but without active ingredients or additives.

Measurement of CO_2
CO_2 was quantified using a Carbon Dioxide Meter with pump-aspirated sampling (Vaisala CARBOCAP® GM70).
For the determination of CO_2 formation rates, the amount of CO_2 produced by 1 g moist beads in 1 h in a volume of 50 mL at room temperature was measured.

For the measurement of CO_2 concentrations in soil, boxes were filled up with a mixture of flower soil and autoclaved sand in a ratio of 1:2 (w/w). 10 g CO_2 releasing beads were placed in 8 cm depth in the middle of each box. The soil humidity was periodically adjusted to 40 % (w/w) and the boxes were kept at room temperature (22±2 °C). CO_2 concentrations and gradients in soil were measured using a drain tube connected to the pump which was inserted into the soil. For simple CO_2 analyses small boxes were used and the drain tube was inserted directly to the CO_2 releasing beads. For the detection of CO_2 gradients, oblong boxes and varying sampling positions were used.

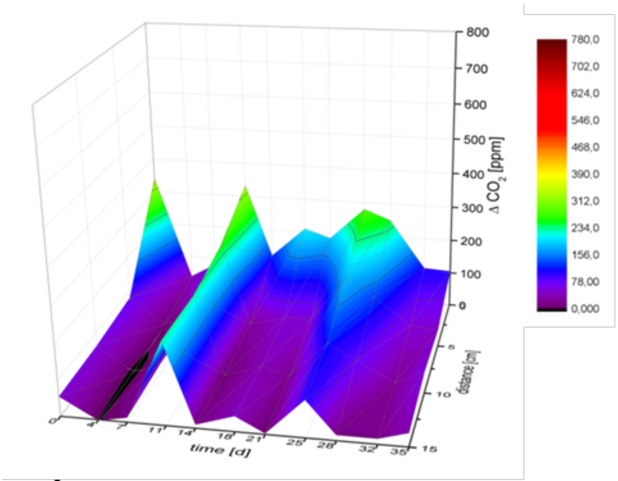
RESULTS AND DISCUSSION

We investigated the influence of different additives on the amount and the duration of CO_2 release with regard to the formation of CO_2 gradients in soil:

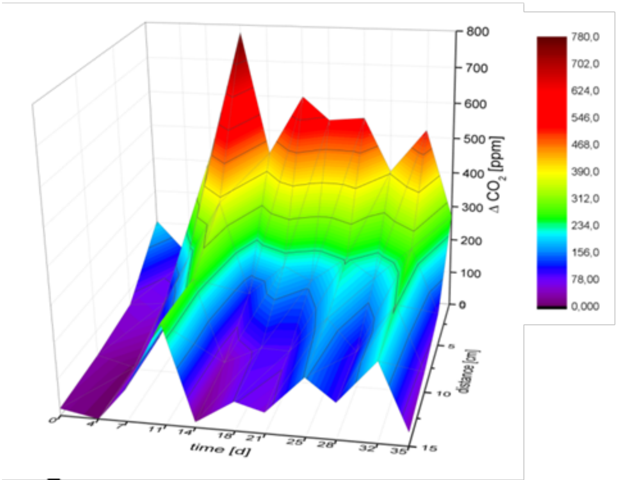
CO_2 release of encapsulated commercial baker's yeast mixture
In sterile Greiner tubes, experiments have shown a CO_2 release over two weeks for encapsulated commercial baker's yeast mixture. In soil, there are detectable gradients over several weeks (Fig. 2 A).

CO_2 release of encapsulated commercial baker's yeast mixture and nutrients
In sterile Greiner tubes, the CO_2 release could be increased by starch containing nutrients with inconsistent results. In soil, the release could

significantly be increased by starch-containing nutrients leading to higher gradients (Fig. 2 B).



A Moist Ca-alginate beads with commercial baker's yeast mixture (16,7 %)



B Moist Ca-alginate beads with commercial baker's yeast mixture (16,7 %) and corn starch (20 %)

Figure 2: CO_2 gradients (ΔCO_2 over distance) in soil over time. ΔCO_2 refers to soil with moist Ca-alginate beads without active ingredients.

As *S. cerevisiae* is not able to metabolize starch, the encapsulated cells benefit from microbial contaminations in the yeast mixture or from soil microorganisms which provide amylases that catalyse the breakdown of starch into sugars.

CO_2 release of pure S. cerevisiae, nutrients and a provider for amylase

To guarantee a better reproducibility and the independence from microbial contaminations or soil microorganisms a pure *S. cerevisiae* culture was used in combination with starch containing nutrients and a synergistic entomopathogenic fungus as an amylase source (Fig. 3). By this way, the CO_2 release could be increased and prolonged over 6 weeks and, at the same time, by means of the fungus, an insecticidal component was incorporated into the bead.

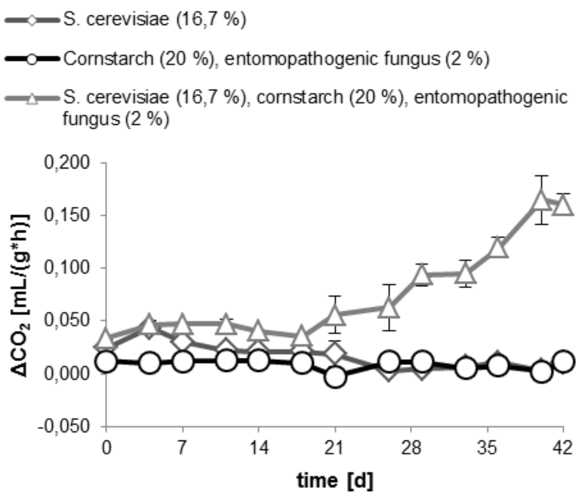


Figure 3: CO_2 formation rates over time. ΔCO_2 refers to moist Ca-alginate beads without active ingredients.

CONCLUSIONS AND OUTLOOK

Experiments have shown a significant CO_2 emission and CO_2 gradients in soil over several weeks. Results indicate that encapsulation methods based on hydrogels may be used for the construction of slow release systems for attractants based on CO_2. First experiments show an attractive effect of these beads (Schumann 2013).

The preliminary findings lay the foundation for the development of novel plant protection products following an "attract-and-kill strategy". Based on the promising results the project ATTRACT targets the development of an innovative formulation based on CO_2 emitting sources and an environmentally friendly "kill" component. Regarding the "kill" component, the project ATTRACT is focused on plant-based environmentally friendly insecticidal substances, such as neem and quassin, which will be co-encapsulated in multiphase or multilayer systems with additives.

REFERENCES

- Schumann et al. (2013) *An encapsulated carbon dioxide source attracts western corn larvae in the presence of maize roots - implications for an attract and kill approach* (submitted)
- Vemmer et al. (2011) *Development of CO_2 releasing beads to control soil borne insect pests - first results*. in *XIX International Conference on Bioencapsulation* (Bioencapsulation Research Group - 5.-8.10.2011 – Amboise) pp 240-241.

[1] *University of Applied Sciences, Department of Engineering and Mathematics, Engineering and Alternative Fuels, 33602 Bielefeld, Germany*

[2] *BIOCARE GmbH, Dorfstr. 4, 37574 Einbeck, Germany*