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# SEMIOCHEMICAL AUGMENTATION IN ARTIFICIAL BLOOD BAIT: A NOVEL STRATEGY AGAINST STABLE FLIES

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**Abstract:** The stable fly (*Stomoxys calcitrans*) is a significant pest affecting not only livestock but also humans, particularly in urban, residential, and coastal areas. Stable flies inflict painful bites, causing discomfort and public health concerns, especially during outbreaks in urban settings and recreational areas such as the Gulf Coast and the Great Lakes in the USA. The presence of stable files in residential areas has also led to legal disputes, zoning challenges, and tensions between agricultural and urban stakeholders. Current control methods, such as the broadcast application of residual insecticides, are increasingly ineffective due to rising insecticide resistance and growing concerns over environmental and human health. Biological control using parasitoid wasps has shown limited success, while visual traps like Alsynite fiberglass and Nzi traps are costly, labor-intensive, and prone to inefficiencies, including non-target captures and frequent replacement needs. To address these challenges, Apex Bait Technologies is developing an innovative semiochemical-based blood substitute liquid bait. This novel bait is designed to exploit the blood-feeding behavior of stable flies, offering a targeted, effective, and environmentally sustainable approach to their control. This bait combines an artificial blood matrix with attractants, phagostimulants, and a reduced-risk insecticide, ensuring minimal impact on non-target species and reducing the application of synthetic insecticides in sensitive urban and ecological areas. This innovation promises to significantly alleviate the stable fly burden on urban populations while providing a practical and scalable solution to a longstanding public health challenge.

**Key words:** Biting Flies, Stable Fly, Artificial Blood Bait, Attract and Kill

## INTRODUCTION

Stable flies are global pests of livestock-related industries, causing significant economic, animal health, and social consequences (Ózsvári 2018; Ose and Hogsette 2014). Traditionally associated with rural animal production systems, stable flies are now increasingly problematic in urban and suburban areas due to rapid urbanization. Their painful biting behavior causes discomfort to humans and animals, leading to economic impacts and social tensions in urban, residential, and coastal regions. These tensions often manifest as lawsuits, zoning restrictions, and conflicts between farmers and housing associations (Mramba 2006, Thomas and Skoda 1993). Current stable fly management strategies include chemical insecticides, baits, traps, biological control, and sterile insect release methods. Among these methods, the broadcast application of residual insecticides remains the most widely employed method for stable fly control in urban areas. However, the emergence of insecticide resistance has significantly reduced the efficacy of chemical controls posing a major challenge to their management (Reissert-Oppermann et al., 2019, Cook 2020). The repeated application of high doses of potent insecticides not only accelerates resistance development but also incurs higher costs and environmental harm, rendering this approach unsustainable for long-term control (Reissert-Oppermann et al., 2019).

To address these limitations, we propose the development and commercialization of a blood substitute bait, an innovative liquid bait formulation containing artificial blood matrix,

semiochemical attractants, and phagostimulants. This innovative formulation aims to provide an effective, environmentally sustainable, and economically viable approach to stable fly management.

## MATERIALS AND METHODS

**Insect:** Stable flies (*Stomoxys calcitrans*) were collected from a livestock farm in California and maintained in the laboratory under controlled conditions (23±2°C, 20-30% RH) with a 12:12 light/dark cycle. Water and sugar water were provided ad libitum, and adults were fed blood once weekly to sustain their physiological needs.

**Insecticide:** Manufacturing-grade pure boric acid, supplied by U.S. Borax, California, was used as the insecticidal component in this study.

**Prototype Bait Matrix:** Apex Bait Technologies, Inc. developed and evaluated a proprietary bait matrix comprising an artificial bait base enhanced with stable fly-specific attractants to lure flies to the bait. The matrix was also supplemented with a specialized phagostimulant to elicit, stimulate, and sustain feeding by the attracted stable flies.

**Lab Efficacy Trial:** The lab efficacy trials were conducted at Apex Bait Technologies' headquarters in Santa Clara, California. Experiments were carried out in BugDorm-2S120 insect cages (60 x 60 x 60 cm). Each cage housed 25-30 stable flies and provided 10% sugar water *ad libitum*. After a 24-hour acclimatization period, a single treatment was introduced into each cage. Mortality was assessed on days one and two by counting the number of dead and live flies. Throughout the experiment, stable fly mortality in control groups was maintained below 10% to ensure validity.

**Treatment:** Four treatments were tested: a blank prototype bait matrix (PBM) without boric acid served as the control, while boric acid was incorporated into the PBM at concentrations of 0.1%, 0.5%, and 1.0% for the other three treatments.

**Data Analysis:** The experiment followed a completely randomized design with four treatments and five replicates per treatment. Each replicate included 25-30 stable flies. Data were analyzed using a two-way ANOVA to evaluate differences in mortality across treatments and replicates.

## RESULTS

The laboratory efficacy bioassay demonstrated that the prototype artificial blood bait with boric acid was highly effective against stable flies, achieving >95% mortality within two days at all tested concentrations (Figure 1). Specifically, the bait containing 2.5% boric acid caused >75% mortality within one day and >95% within two days, outperforming both the 1% and 5% boric acid formulations in terms of rapidity of kill. The baits with 1% and 5% boric acid also reached >95% mortality, though requiring three days to do so. These results highlight the compatibility of boric acid as an insecticide in the artificial blood bait matrix and suggest that the 2.5% concentration offers an optimal balance between efficacy and speed of kill. Further, the consistent performance across concentrations underscores the robustness of the artificial blood bait formulation over sugar attract and kill baits.

## DISCUSSION

The results indicate that the PBM with 2.5% boric acid achieved >75% mortality within the first day, outperforming the PBM treatments with 1% and 5% boric acid. This could be attributed to two factors: the 5% boric acid may act as a feeding deterrent, leading to reduced feeding rates by stable flies, while the 1% concentration might be too low to elicit rapid mortality. By day 2,

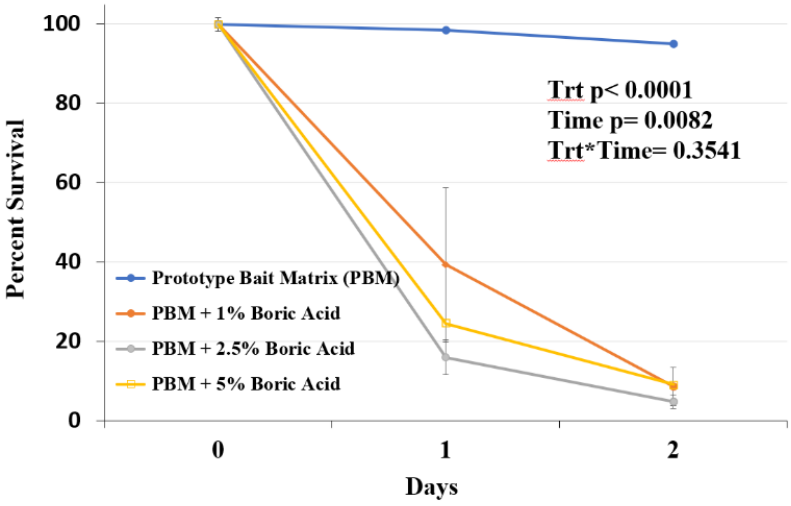


**Figure 1**. **Stable fly survival after feeding on toxic bait.** 1%, 2.5%, and 5% of boric acid in bait matrix result in <10% survival (>90% mortality) within 2 days. Each treatment had 5 replicates. Each Replicate had 25-30 stable flies. Marks represent mean ± SE. Data were analyzed using two-way ANOVA.

however, all treatments resulted in similar high mortality rates, exceeding 95%. This suggests that over time, the differences in the effectiveness of low and high boric acid concentrations diminish. Nevertheless, given its rapid efficacy within the critical first 24 hours, the 2.5% boric acid concentration appears to be the optimal choice for our artificial blood bait formulation. Future studies could explore the feeding behavior and physiological responses of stable flies to varying boric acid concentrations in the field setting to further refine this observation.

## CONCLUSIONS

This study establishes the feasibility of using artificial blood bait with 2.5% boric acid for stable fly control, achieving over 95% mortality within two days in laboratory trials. The results exceed EPA efficacy standards for livestock bait products and provide strong support for the continued development of this technology. The promising laboratory and field data strongly justify future large-scale field trials to evaluate the bait's effectiveness in diverse environments, including the urban areas experiencing outbreaks of stable flies and other biting flies. Such trials will also allow for the assessment of off-target impacts to ensure safe and sustainable implementation.

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