Effect of Soil Water Content on Toxicity of Fipronil Against *Solenopsis invicta*

by

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ABSTRACT

This study evaluated the effect of Fipronil on the survival of fire ant workers with different doses and soil water contents and further examined the persistent effect of the same dose of powder at 10%, 50% and 90% soil water content. The results showed that mortality was positively correlated to the dosage. This result indicated that the survival rates of workers treated by powder at different RSW (Relative soil water content) were significantly different ($P < 0.01$). At the RSW of 10% and 20%, the survival rates of workers were 40.67 and 49.00 respectively, which showed no obvious difference from other treatments but were lower than the control. The survival rate decreased sharply when the RSW was 90%, and was obviously lower than that of treatments at moderate (30-50%) RSW. The contact powder showed worst persistent effect when the soil water content was 10%, but at the soil water content of 50% and 90%, the lethal effect of the powder was higher and was more persistent.

Key words: *Solenopsis invicta*, Contact powder, Soil water content, Mortality rate, Persistent effect

INTRODUCTION

The Red Imported Fire Ant, *Solenopsis invicta* Buren, is a dangerous pest native to sub-Amazonian South America, and invaded the southern United States due to quarantine negligence in the early twentieth century. *S. invicta* feeds variously, breeds rapidly, has ferocious and competitive habits, and does harm to human health, public safety, agriculture and forestry production and the ecological environment of the invaded region. It is listed as one of the world's most dangerous 100 invasive pests (Zeng *et al.* 2005). At the end of

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2004 serious harm caused by *S. invicta* was found in Wuchuan, Guangdong Province which marked the successful invasion and colonization of this dangerous pest in China (Zeng *et al.* 2005).

Ants are distributed in tropical and subtropical regions, where there are frequent rainfalls all year round, especially in the humid and rainy spring. Spring and summer are the active breeding seasons for *S. invicta*. Rainfall will not reduce the activity of fire ants, but induces nest moving and division for the ants, which will lead to the rapid increase in the number of fire ant nests (Zhao *et al.* 2009). Frequent rain also causes some difficulties for prevention and treatment, thus the traditional prevention work will not be conducted in this season. However, if the prevention and control work of this period are not maintained, *S. invicta* will thrive and create favorable conditions for expansion, which will add to the huge challenge of fire ant prevention and control.

Large-scale application of baits to control *S. invicta* is the most effective method. Bait can help control *S. invicta* at a low level chronically (Banks 1990). To take full advantage of bait and pesticides in the control of *S. invicta* in urban areas, a two-step method was recommended: First, baiting is used for large area processing, and then contact toxicity of insecticides is employed to deal with the remnants of ant mounds individually (Drees *et al.* 2000). Contact insecticides are commonly used in controlling red imported fire ants by individual mound treatment s(Chen 2006; Appel & Woody 1990). Fipronil was one of the most popular contact powders introduced for the control of *S. invicta* (Sparks & Diffie 1998; Collins & Callcott 1998; Barr & Best 2003), and was considered the ideal pesticide for *S. invicta* in frequent irrigation areas (Greenberg *et al.* 2003). However, in the rainy spring and summer, rain erosion, soaking and humidity makes pharmaceutical degradation failure occur more frequently (Krushelnycky *et al.* 2005).

After each rainfall newly repaired nests are clearly identifiable, which may be convenient for the prevention and treatment of *S. invicta* in spring and summer, combined with the use of moisture-proof types of bait. It was reported that Fipronil proved to have greater efficacy in the appropriate soil moisture conditions than dry soil conditions (Zhuang *et al.* 2007). Therefore, a full understanding about the effect of the amount of soil water content on contact powder efficiency will help to facilitate fire ant control techniques after rainfalls in spring and summer.

MATERIALS AND METHODS

Insects and tested powder

S. invicta workers were randomly collected from ten polygyne colonies in the campus of South China Agricultural University. The ants were analyzed within 2 weeks of capture. The social form of *S. invicta* was confirmed by the number of queens present in each colony (Porter 1992). More than two queens were found in each polygyne colony. Ants were collected directly from the mounds with a gardening trowel and placed in plastic boxes. The upper inner edge of each box was lined with talcum powder to prevent escape. The collected ants were fed with a mixture of 10% honey and live insects (*Tenebrio molitor* L.). A test tube (25mm×200 mm), which was filled partially with water and plugged with cotton, was used as a water source. Ants were maintained in the laboratory at 25 ± 2°C.

Contact powder (0.1% Fipronil) was provided by entomology institute of Guangdong, China.

Calculation of the soil water content for powder-soil mixture

Sandy soil was selected for the bioassay and was heated for 24 h at a temperature of 100°C. The formula for the calculation of soil water content was as follows:

Relative soil water content (RSW) =Soil actual water content (SAW)/ Soil saturated water content (SSW)×100%:

To prepare the soil with different RSW for powder-soil mixture, 500 g of sand plus powder was placed into plastic sample boxes (20 cm×15cm×15cm), and water was mixed with the sand according to the formula. Before testing, there was a 5 min wait for the water to fully penetrate the sand.

Lethal effect of contact powder on *S. invcta* workers at different dose

Glass jars (6.5cm bottom diameter, 5.0cm diameter, 12cm height) were filled with the soil with 50% relative water content which contained contact powder against the fire ants. Powder contents of 0.02g/150g sand, 0.08g/150g sand, 0.14g/150g sand, 0.20g/150g sand, and 0.26g/150g sand were used for the test. 100 worker ants were put into each jar, whose edge was wiped with talcum powder to prevent escape of workers. Five repeated tests were conducted for each dose. The drug content for the control was 0. We checked the mortality of workers and recorded the data 24 hours after the workers were put in.

Lethal effect of contact powder on *S. invcta* workers at different RSW

Different glass jars were filled with soil whose powder content was 0.26g/150g sand, and the relative water contents of the soil were 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% for the test. 100 worker ants were put into each jar, whose edge was wiped with talcum powder to prevent escape of workers. Five repeated tests were conducted for each RSW. Two controls were set and the drug content was 0 while the relative water contents were 10% and 90% respectively. We checked the mortality of workers and recorded the data 24 hours after the workers were put in.

Persistent lethal effect of the contact powder on *S. invcta* workers

Soil with relative water contents of 10%, 50%, 90% and powder content of 0.26g/150g net sand was prepared in glass jars. The jars were sealed with plastic film to prevent excessive evaporation of water, and the date labels were tagged. 5 replicates were conducted for each RSW. These soil preparation steps were exactly followed for five days for the following bioassay.

100 worker ants were put into each of the glass jars with prepared soil, and the edge of talcum powder was wiped to prevent escape on the last day. We checked the mortality of workers and recorded the data 24 hours after the workers were put in.

Statistical analysis

Variations in the survival of *S. invicta* with varied RSW were analyzed using analysis of variance. T-test for paired data was used to compare the mortality of workers at different RSW (between 50% and 10% or 90%). Linear regression was used to determine the relationship between the survival and powder's weight. All the statistical analyses were conducted using the SPSS13.0 software package.

RESULTS

Lethal effect of contact powder on *S. invcta* workers at different dose

The results indicated a strong correlation between the survival rate and powder's concentration when RSW was 50% (Fig. 1). The survival rates of the workers (Y) in the powder treated soil were best predicted by fitting the linear model:

$Y=-14.38X+107.02$ (n=5, R=0.98625)

In this model, X signifies the powder's concentration. The survival rates were significantly and positively correlated to the powder's concentration ($P<0.01$).

Lethal effect of contact powder on *S. invcta* workers at different RSW

This result indicated that the survivals of worker treated by powder at different RSW were significantly different ($P<0.01$). When the RSW was 10% and 20%, survival of workers was 40.67 and 49.00 respectively, which were not obviously different from other treatments but lower than the control. The survival rate decreased sharply when the RSW was 90%, and was obviously lower than that of treatments at moderate (30-50%) RSW (Table 1).

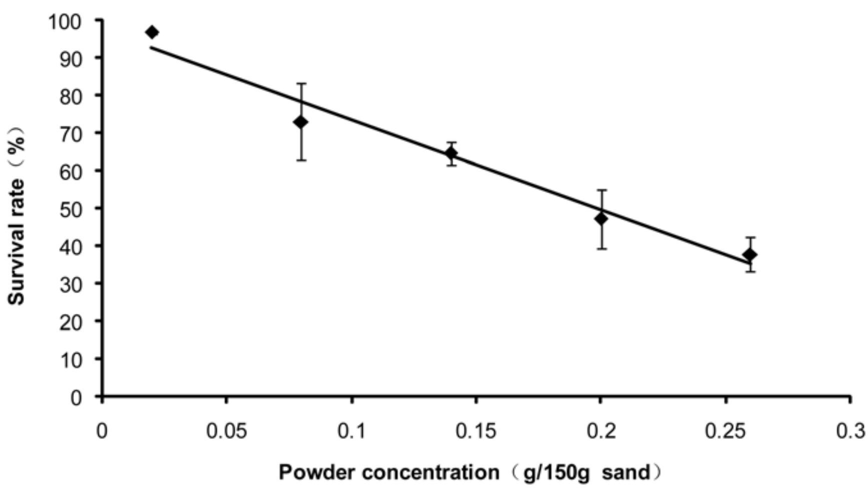

Fig. 1 Lethal effect of contact powder on *S. invcta* workers at different dose

Persistent lethal effect of the contact powder on *S. invcta* workers

The result confirmed that the control efficacy of powder was stronger under the condition of the high (50% or 90%) RSW compared with the low (10%) RSW (For 50% RSW: t=-4.610, p=0.006; for 90% RSW: t=-7.201, P=0.001). In addition, the mortality of workers was lower than 10% after 2 days' treatment at low (10%) RSW. While at high (50% or 90%) RSW, the mortality of workers was higher than 80% after 2 days' treatment (Fig. 2).

Table 1. Lethal effect of contact powder on *S. invcta* workers at different RSW.

RSW	n	Survival (Mean± SE)
10%	5	40.67±4.57bc
20%	5	49.00±3.81bc
30%	5	65.00±7.70c
40%	5	63.00±1.46c
50%	5	64.33±4.39c
60%	5	51.67±8.33bc
70%	5	29.00±10.22bc
80%	5	23.67±13.57bc
90%	5	21.67±1.52b
CK 1	5	99.00±0.67a
CK 2	5	97.00±1.34a

CK1 and CK2 mean no drug content while the relative water contents were 10% and 90% respectively. Means in the same column followed by the same small letter are not significantly different (LSD) at the level of 0.05

DISCUSSION

Chemical control of *S. invicta* is easily affected by moist conditions and rainfall because the bait formulations have a propensity to degrade when wetted (Kafle *et al.* 2009). Therefore, the development of fire ant baits that

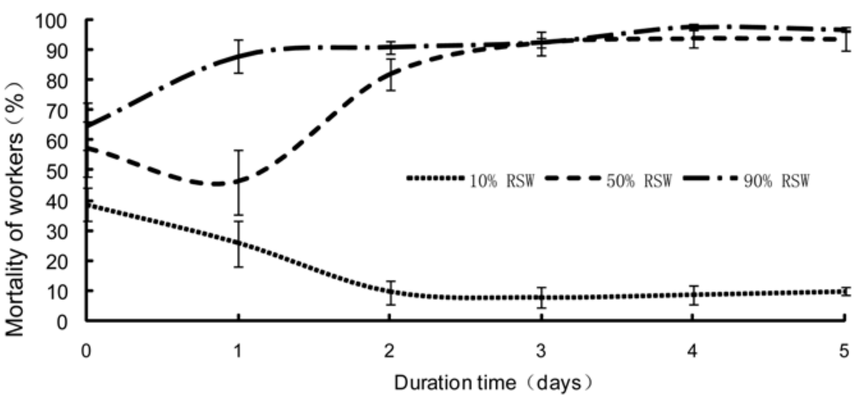

Fig. 2 Persistent lethal effect of the contact powder on *S. invcta* workers

are resistant to high humidity or water can increase the efficacy of chemical control (Kafle *et al.* 2010). In this study, we observed that the moist soil condition may be helpful to the powder control effect.

With the same relative water content in the soil, the amount of drug powder has a linear correlation with its lethal effect. When the relative water content in the soil is higher than 70%, the net powder showed a better lethal effect at the same dose, while the lethal effect becomes poor at the relatively low water content in soil (10% for example). When the dose is 0.26g/150g sand, the net lethal effect is almost lost and the worker mortality dropped to about 10% two days after the red ant powder trial was conducted in the indoor conditions. When the worker ants were directly exposed to the powder of 0.1% fipronil, the median lethal time for the workers was 20.06 h (Zhuang *et al.* 2007). As our tests found, when the red ant powder was mixed with higher relative water content (50%) of the soil, the powder achieved the best efficacy two days later. We also found that the mortality rate of the workers is relatively high in the dry soil, probably because low humidity conditions are not suitable for the survival of the red imported fire ants. Drought has a certain impact on *S. invicta* reproduction and spread. In addition, extreme humidity like rainfall will reduce 40% of ants' foraging activity. Rainfall will block the underground channels and interfere with pheromones, which will also affect recruitment for the fire ants (Porter & Tschinkel 1987).

A prior study ruled out temperature factors on the survival rate of *S. invicta* (Porter 1988), At constant temperature conditions, the soil moisture content and the amount of red powder have a great impact on the mortality of ant workers (Hadley 1994). Of course, these conclusions are drawn according to indoor experiments, so further verifications by field trials are needed. For social prevention and treatment of invasive insects like the red imported fire ant (Gentz 2009), the use of bait (Levy *et al.* 1974; Williams 1983) and *Beauveria bassiana* (Stimac *et al.* 1993) were also employed in addition to the powder, among which the bait was more vulnerable to moisture, and the fungi can play a more significant control role in high humidity conditions. Therefore, further studies should be carried out about the combined effects of powder and fungi in high humidity conditions, and the bait's water-proof efficiency could be improved, which would significantly improve fire ant control techniques during the spring and summer rainy season.

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