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Quick knockdown results in high mortality: is this theory correct? A case study with phosphine and the red flour beetle

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Abstract

BACKGROUND: The fumigant phosphine is used all over the world for disinfestation of stored grains and commodities. Adults of 23 different populations of *Tribolium castaneum* from 10 different countries, were evaluated for phosphine resistance using a modification of Detia Degesch Phosphine Tolerance Test Kit (DDPTTK). Adults were exposed to 3,000 ppm and recorded for 5 to 270 min for their mobility.

RESULTS: Among the tested populations, high level of phosphine resistance was recorded from populations of Brazil, Serbia, and Spain. No survivals were recorded after 7 days post exposure for 8 out of 23 population tested.

CONCLUSIONS: Our work showed four scenarios: 1. quick knockdown - low (or no) recovery; 2. Slow knockdown – high recovery; 3. Quick knockdown - high recovery and 4. Slow knockdown – low recovery. Our data indicate that post exposure period is critical for the evaluation and characterization of phosphine resistance.

Keywords: knockdown; mortality; resistance; phosphine; stored product insects; *Tribolium castaneum*

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1. INTRODUCTION

Phosphine (PH3) is the most widely used insecticide for the control of stored product insects worldwide [1,2]. The use of phosphine has been proven an effective way to disinfest stored products in various types of facilities and commodities, and against a wide range of stored product insect species [1,3-5]. Nevertheless, the continuous use of this fumigant has led to the development of resistance, which is now widespread in many parts of the world, a development of alarming dimensions and threatens global food security [6,7]. In fact, many of the major stored product insect species have been found to be resistant to phosphine, such as the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) [8], the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [9], the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostryhidae) [10], the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) [11], the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) [12] and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) [13]. For the species, above, numerous resistant strains have been selected for further studies from different parts of the world, such as US [5,14], China [15], Morocco [4], Turkey [16], Brazil [12], Australia [7] and Greece [17].

The diagnosis and characterization of phosphine resistance has been studied thoroughly by many research groups, which proposed different, and often contradictory evaluation protocols [8, 9,18,19]. The most widely adopted protocol is the Food and Agriculture Organization (FAO) method number 16 [20]. This method uses different concentrations according to the LC99 of different stored product insects after 20h of exposure, and has a following evaluation interval 14 days later, to test delayed mortality or recovery [12,21]. In this context, mortality patterns at this delayed interval can be used to quantify resistance. However, the method is laborious and cannot be used on site by the grain managers during phosphine fumigation practices.

A quick test that has been recently updated is the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK, Detia Degesch GbmH, Germany) [17,22,23]. This test is operated at

3000 ppm and a fixed period of exposure time in minutes [22-24], and characterizes the susceptibility of the populations according to time that is required for knockdown of the exposed individuals, which is usually 14 minutes or less [24]. This kit can be easily operated on site, and can be utilized to draw quick fumigation decisions and to guide resistance mitigation measures. In recent work, Agrafioti et al. [17] found that the use of DDPTTK gave reliable results for a wide number of stored product beetle species and populations that had been collected from Greece, and that this quick diagnosis could be further utilized in resistance screening programs for quick detection and estimation. Still, DDPTTK is based exclusively on the speed to knockdown, i.e. how many minutes are required for insects to exhibit deviations from normal movement when exposed to 3000 ppm of phosphine, and does not predict if the speed to knockdown means, eventually, increased mortality. In this regard, Nayak et al. [25] showed that knockdown patterns after a certain period of time (hours), could distinguish susceptible, weakly resistant and strongly resistant strains of *C. ferrugineus*. Still, even in that case, the evaluation solely relied on mobility, without the estimation of mortality after the termination of the exposure. Theoretically, knockdown may not be a good indicator of the concomitant mortality, as quick knockdown, at least in the case of some insecticides, is related with reduced uptake of the toxic agent, and may lead to increased survival [26]. For instance, when insects become immobilized at low phosphine concentrations at short exposures there are some immobilization thresholds in which insects can experience short survival interval as they are protected from the effects of phosphine [26,27]. This may be also true in the case of quick diagnostics to phosphine that are based on short exposures to high concentrations, which lead to quick knockdown and rapid immobilization, with uncertain correlation with predicted mortality [23]. Hence, the relationship between “time-to-knockdown” and “time-to-mortality” has not been thoroughly examined so far for phosphine, despite the fact that, eventually, mortality, and not knockdown, is the desiredoutcome of exposure. Under this theory, if there is a potential positive correlation between these two intervals (i.e. knockdown and mortality), then immobilization patterns can be used for mortality predictions, and, to a certain extent, as tolerance/resistance indicators [25,28]. This theory is based on an “equilibrium” of three parameters, as expected, and not observed, mortality can be used as indicator

of resistance, just like immobilization can be used to predict mortality. The overall hypothesis has not been evaluated thoroughly so far, despite the fact that there are some scattered information that indicate both positive and negative results of this "equilibrium" [23,26,27].

Using genetic methods, researchers can identify the presence of resistant genes that are associated with phosphine resistance. Schlipalius et al. [29] identified the mutations that are related with phosphine resistance in the DLD gene (dihydrolipoamide dehydrogenase) in the rph2 locus. More recently, Schlipalius et al. [30] described an innovative method to detect phosphine resistance in the DLD gene and estimate the distribution and frequency of resistance in *R. dominica* using many individuals in a single run. Similarly, Nguyen et al. [11] investigated a unique amino acid substitution (N505T) in the DLD gene in strongly resistant *S. oryzae* populations. Chen et al. [18] using the cleaved amplified polymorphic sequence method (CAPS) detected the mutations in the DLD gene of *T. castaneum* and *R. dominica*. More recently, Hubhachen et al. [31] compared the mutations of a Brazilian strongly resistant population with a strongly resistant population of US using CAPS markers and found the common P45S mutation but also two additional mutations, G131D and V167A, only in the Brazilian population. Still, these tests are extremely laborious, and cannot be utilized on site for the evaluation of the resistance status of the insects that are to be treated commercially.

Tribolium castaneum, is a model species for different areas of scientific research [32,33]. Being the first agricultural pest had its genome totally sequenced [34]. *Tribolium castaneum* has been used several times for molecular and biochemical studies, including studies that are focused on resistance to phosphine [18,31,35]. At the same time, this species has been thoroughly used in biology and ecology modelling, as it can be easily reared under laboratory conditions, and has a global distribution. *Tribolium castaneum* is a major pest of durable stored products, able to cause serious losses and quality degradation [36,37]. Populations of this species that are resistant to phosphine have been found in many parts of the world, and it is considered among to the most studied species in this respect [9,18,28,38]. For example, Cato et al. [14] tested 24 populations of *T. castaneum* from North America

and characterized 12 of them as resistant to phosphine. This species has been thoroughly evaluated for phosphine resistance at the molecular level, which identification in the identification of specific mutations and markers that can be used to separate resistant from susceptible populations, as well as strong from weak resistance [18,28,38].

Regarding the comparison of resistance evaluation with traditional diagnostics, there are numerous studies that have focused on either FAO [5,39,40], DDPTTK [22-24,40] or both [17,19]. Recently, Agrafioti et al. [17] found that knockdown levels after short exposures to DDPTTK were positively correlated with 7-d mortality results from the FAO tests, and concluded that immediate knockdown can be correlated with delayed mortality, in most of the cases examined. However, in that study, although the authors find good correlation between knockdown and mortality for most species tested, correlation was poor for the populations of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), which suggests that this relationship is population-dependent, rather than a "global phenomenon" [17]. Athanassiou et al. [24] exposed two populations of *T. castaneum*, one susceptible and one resistant to phosphine, to DDPTTK for 15 and 90 min, and found different post-exposure recovery patterns between the two populations, which was not always correlated with immediate knockdown during the exposure interval. In that work, knockdown lead to increased delayed mortality only for the susceptible population, while the majority of the individuals of the resistant populations fully recovered, regardless of their knockdown percentages [24]. Based on the above, immediate responses could be regarded as a predictor of delayed effects, mostly on a "time to immobilization" pattern, rather than a immobilization percentage at a fixed exposure interval. Nevertheless, there are still serious data gaps that prevent generalizations of the use of a robust "time to immobilization" resistance indicator.

The aim of the current work is to examine the basis of the theory "quick knockdown= high mortality" after exposure to phosphine, by using different populations of *T. castaneum*. For this purpose, we have collected different populations of *T. castaneum* from different parts of the world, which were evaluated and then separated for their susceptibility to phosphine [17,22,42]. Our basic expectation from this study was to

be able to show that a quick diagnostic, on which the "verdict" can be available in minutes, can be used for the prediction of tolerance/resistance, without the need to follow time consuming bioassays, which last for days, and may have no practical importance in "real world" applications. If the above theory is correct, the short-term diagnosis can be then used as a quick resistance diagnostic, when time limitations are critical in stored product insect management programs. Apart from just a screening for resistance, some of the populations of *T. castaneum* that were used here have been tested already for mutations that are related with strong resistance and hence, the populations used cover a wide range of tolerance/resistance levels [43,44,31].

2. MATERIALS AND METHODS

2.1 Insects

Twenty-three populations of *T. castaneum* from different geographic locations throughout the world were evaluated for these tests (Table 1). All populations were reared in the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production and Rural Environment, University of Thessaly for at least 2 generations. Rearing was carried out in glass jars containing white flour, in incubators set at 25 ± 1^{o}C, 60 ± 5% relative humidity (r.h.) and continuous darkness.

2.2 Phosphine Tolerance Test

We used DDPTTK as suggested by Athanassiou et al. [23] and Sakka et al. [19], following some modifications. Phosphine was produced in a plastic cubic canister, by using the standard DDPTTK tablets and 50 ml of water, and the required amount of phosphine was removed with a 100 ml syringe, to reach the desired concentration [19]. Twenty adults of each strain were placed in the syringe of the kit (separate syringes for each strain), and exposed to 3,000 ppm of phosphine for different intervals, i.e. 5, 10, 15, 20, 25, 30, 45, 60 and 90 minutes, and after this, every 30 minutes until the completion of 270 minutes. After each exposure interval, active, knocked down and immobilized insects were recorded, and after the last exposure interval, all insects (active, knocked down and immobilized) were transferred to clean petri dishes with flour (approximately 0.5gr) for an additional period of 7 days, to record the delayed mortality or recovery. The definitions for active, knocked down

and immobilized adults are given by Athanassiou et al. [23]. We classified the knocked down as quick knocked down (less than 90 minutes) and slow knocked down (until 270 minutes). Briefly, active insects were the ones that indicated normally coordinated movement, knocked down were the ones that showed deviations from normal movement, and immobilized the ones that had no visible movement. Phosphine concentration was measured with quantitative gas chromatography (GC) using a Shimadzu GC-2010Plus (Shimadzu, Kyoto, Japan) instrument equipped with a GS-Q column (30 m long × 0.25 mm i.d., 0.25 µm film thickness, MEGA S.r.l., Italy) and a flame photometric detector set in the phosphorous mode as suggested by Cato et al., 2017, and also through glass tubes (Draeger 25A, Draeger Safety AG & Co., Germany). The entire procedure was carried out in 3 sub-replicates (syringes) and 3 replicates (canisters), with new phosphine production for each replicate. Control syringes (without phosphine) were used parallel to the trials.

2.3 Statistical analysis

Longitudinal measurements were treated using Generalized Estimating Equations (GEE) modeling in order to assess differences between strains and properly accommodate the variable number of time points per strain. Furthermore, standard regression modeling was employed for the assessment of linear relationships between variables of interest with a quantitative outcome, while depiction of results included bubble plots for the direct depiction of relationships between three quantitative variables and ternary plots. The latter were selected because of the nature of the outcome counts in triplets of (alive, knocked-down, dead) adding up to a known total. The test-wise significance level was set at 0.05. Stata 17.0 (Stata Corp., College Station, TX) and R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria) and the ggtern package were used for data analysis and depiction [45].

3. RESULTS

Complete survival was recorded for all control populations for both immediate and delayed effect. From the populations tested, 3SP18.1, BTS and D1 were found to have adults that were still active after the termination of the 270 minutes interval (Figure 1A). Moreover, knocked down individuals were recorded at the 270-min interval for the populations of 3SP.18.1, BTS and D1 (Figure 1B). In contrast, adults for most populations were immobilized at the 90-min interval (Figure 1C). No immobilization for the population BTS, 3SP.18.1, SM20.8, 4.21.10SP, 4Hun, 6Tur, RB1, RioV, SP16.8 and D1 (Fig 1C).

Based on the results of the 7-day post exposure period, 15 populations were recorded to have active individuals (Figure 2B). The highest percentage of survival was noted for RB1 (Figure 2A,2B). Quick knock down (less than 90 min) with high recovery (more than 90% of active individuals) was recorded for RB1, while quick knock down with no recovery was recorded for 4SRD, Inj, M6 and 12GS. Figure (2B) shows the relationship between active and immobilized adults. Most of the individuals are immobilized after 7 days post exposure and high level of active individuals were recorded for 3SP18.1, RB1, BTS, 3SP18.1, RioV and D1 (Figure 2). The GEE analysis showed significant differences ($P<0.001$) among populations regarding the changes of

active/immobilized/knocked down adults throughout the exposure time (Figure 2B). Moreover, Figure 2 (C) showed that adults of populations 3SP.18.1, BTS and RB1 are mostly active in contrast D1 which is in the center of the ternary and not clear which category of adults can be placed; in contrast individuals in all populations are nearly all immobilized.

4. DISCUSSION

The aim of the study was to investigate phosphine resistance in *T. castaneum* populations from different parts of the world, by using the DDPTTK as a quick diagnostic. Based on our results, by using 90 min as the time threshold eight populations out of the 23 tested here can be characterized as tolerant/resistant to phosphine. This is proportional to the populations of these species that were found to be resistant during different population surveys in many other countries [6,17,41,43,46].

One of the fundamental differences between the quick diagnostic protocols and the "classical" dose bioassay methods FAO [4,12,20] is that in the case of the quick diagnostics there is no "delayed" post-exposure evaluation period, and a decision is made during exposure. Hence, for the DDPTTK characterization of the insects is performed between 9 and 14 minutes, depending on the target species [23]. Similarly, Nayak et al. [25] used knockdown as an indicator of resistance for *C. ferrugineus*, and underlined that if there were adults that were still active after approximately 5 hours, the population should be regarded as strongly resistant to phosphine. In a recent work, Nayak et al. [47] developed a quick knockdown test against *S. oryzae* and found that adults of the susceptible, weakly and strongly resistant populations needed 12.52, 167.9 and 1.510 min to knockdown, respectively. The studies above indirectly indicate that there is a correlation of immediate responses of the insects during exposure with delayed effects, i.e. mortality or recovery days later, and consider that these delayed effects can partially simulate "real world" fumigations. According to the protocol followed, these delayed effects could range from some days [9,23] to two weeks [5], and relies on the principle that the majority of the insects that are

exposed to phosphine can exhibit some type of deviation from normal movement. However, these deviations may have a different outcome depending on the population, i.e. delayed mortality in the case of susceptible populations and recovery in the case of resistant populations. The theory of the correlation of immediate with delayed effects has been practically proved for many stored product insect species in the case of contact insecticides. For example Baliota et al. [48] evaluated thiamethoxam, chlorfenapyr and lambda-cyhalothrin against adults of *S. oryzae*, the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). They found that knock down more likely results in mortality than recovery. In that study, the authors found that in short exposures (first day) knockdown was high for adults that had been exposed to either thiamethoxam or lambda-cyhalothrin which resulted in a considerable delayed mortality some days later [48]. Similarly, Arthur [49] tested the efficacy of diatomaceous earth (DE) against *O. surinamensis* after 4–72 h of exposure and recorded a considerable post-exposure mortality. Nevertheless, the same theory has been questioned in the case of phosphine, due to the immediate "narcosis" of the exposed individuals that may affect the insecticidal efficacy of the gas only temporarily [26,41]. Athanassiou et al. [24] by using two *T. castaneum* populations, one susceptible and one resistant to phosphine, found that time to immobilization was highly correlated with delayed effects 7 days after exposure. In that study, the authors found that the resistant *T. castaneum* adults that had low percentages of immobilization during a 90-minute observation period of exposure to 1000 or 3000 ppm of phosphine had an extremely high recovery 7 days later. In contrast, for the susceptible *T. castaneum* population, adults were rapidly immobilized during exposure, and their post-exposure mortality percentages were close to 100 % [23]. Agrafioti et al. [17] was the first that successfully tested the correlation of the quick diagnostic knockdown results and mortality after long exposures, and found that there was a significant and positive correlation between these two parameters. In that work, the authors evaluated 53 strains of stored product beetle species, and found that the results of the immediate knockdown after quick exposure to DDPTTK, could be correlated well with the 7-day

mortality results after exposure on 30 ppm for 20 hours, according to the FAO evaluation protocol [17].

Our data examined the delayed mortality or recovery at the 7 day post-exposure period that follows the quick diagnostic, and clearly underline that the "speed to knockdown" could be used to predict delayed mortality after exposure to phosphine. The current data set is qualitatively different than that of delayed effects after exposure for relatively long periods (20 hours or longer) that have been examined in previous studies in low or moderate concentrations [8,17,50], as we examined the delayed effects after extremely short exposures (minutes) at an elevated concentration (3,000 ppm). In light of our findings, the delayed effects after these short exposures can be used for prediction of mortality, in the same way that this theory occurs in the case of longer exposures at lower concentrations. In other words, the short exposure knockdown results can be used to estimate the tolerance/resistance of the population that is tested. Moreover, the way that this test is performed, can provide immediate results that can be utilized by fumigators when designing a resistance management strategy on site, without the necessity to follow the long exposure tests that are too laborious and unrealistic to be performed at the industrial level [17,22,28].

Given the positive correlation of the above estimation parameters, our data are in accordance with the theory "quick knockdown=high mortality". However, it becomes evident that additional experimentation is required to define the "break points" in such an equation. Here is to define possible "break points" levels of resistance, like weakly resistant, from the ones that are strongly resistant. This exercise has been performed by Nayak et al. [25], Nayak et al. [47] and Cato et al. [41], for populations of *C. ferrugineus*, *S. oryzae* and *T. castaneum*, respectively. In contrast, the DDPTTK has been tested to separate populations that were not susceptible to phosphine, by examining the successive knockdown times of laboratory populations of 30 stored product beetle species [23]. In our tests we have examined both categories of resistance, i.e. populations that are weakly resistant and RB1 which is considered as strongly resistant to phosphine [31]. Hence, by converting the quick diagnostic protocols above the separation of weak and strong resistance [41,47], our data for

DDPTTK show that, for the population RB1 had a different behavioral response from the other data. The knocked down time for RB1 was after 5 minutes of exposure and was molecularly detected for the mutations that are related with phosphine resistance by Hubhachen et al. [31] (referred there as TCBR). In that study, the authors found the P45S mutation, which has been recognized as a commonly found resistance-related mutation, but also two additional mutations, G131D and V167A, and merit additional investigation towards this direction [31]. Results from our study suggest that some resistant populations behave as "susceptible" and a quick test cannot be able to "predict" the resistance status. RB1 population is an exception to the 'rule' and it becomes a challenge for a quick diagnostic test. In addition, the diagnosis between weak and strongly resistant populations that is globally accepted is required to improve DDPTTK.

If the above theory stating that quick knockdown results in high mortality is correct then our data clearly show that there are three additional populations, BTS, 3SP18.1 and D1 that can be also considered as strongly resistant. In fact, for these populations, there were adults that were still alive even after the termination of the observation period (270 min). Based on the above, the quantification of knockdown is not an easy task, as it is based on rather observer-related results [24]. Hence, the definitions of immobilized, knocked down and active may be perceived in a different way according to the person that takes the measurements, that is, it is subjective. This is why Athanassiou et al. [24] based their classification of resistance on two fundamental insect behavioral observations: normal movement and deviations from normal movement, and relied mostly at the time to knockdown, rather that the qualitative characteristics of knockdown per se. However, the speed on which the adult beetles enter to the knockdown stage varies even among the susceptible populations. Concurrently, there are strains that do not register 100 % knockdown, which also have high survival rates at the post exposure period. Nevertheless, our data show that this relationship is not linear, and probably after a certain cut point, does not follow the rule "the later the knockdown the higher the survival". As it is evident from the recovery data, indeed recovery is high for the populations that had increased knockdown time in comparison with others, but survival on these populations was not

proportional to their knockdown times. Further analysis can reveal potential knockdown-recovery equations that can be used towards this direction.

Most of the populations tested here had some adult recovery after the termination of the 7 day post-exposure interval. This is expected, as the exposure period was short (lower than 270 minutes), although individuals were exposed in high concentration (3,000 ppm), , and any knockdown effect could be, at least in part, reversible. With some deviations, the populations that had the longest time to knockdown though were the ones that had the highest percentages of adult survival. The plasticity of this knockdown-survival relationship is remarkable, as it includes many "grey areas", and underlines the temporary effects of short exposures to phosphine. One interesting expression that has been used for this stage is "narcosis", and has been thoroughly described by Winks [26] and Winks and Waterfold [51]. Although narcosis is not used that much by researchers anymore to describe the effects of phosphine, and in most of the papers knockdown is used much more extensively, the outcome of narcosis has not been clarified in detail. Our results show that, for the majority of the adults and the populations tested, even at the short exposure intervals examined, narcosis is far more likely to lead to delayed mortality than to recovery. Considerable deviations from this general rule may indicate resistance to phosphine.

The current protocol shows that more than 50 % of the populations tested here can be considered as tolerant/resistant to phosphine, based on evaluation through DDPTTK. Given that, the two parameters that were tested were knockdown and recovery and hence, we can consider four different combinations: i) quick knockdown- low (or no) recovery, ii) slow knockdown- high recovery, iii) quick knockdown- high recovery and iv) slow knockdown- low recovery. The results of the present study for the different *T. castaneum* populations show that most cases observed in this study fit into scenarios 1 or 2, but our data indicate that post exposure period is crictical. On the other hand, there are some indications that some populations, such as the RB1, may behave in a way that it is better described with the category (iii), while none of the populations tested can be classified in the category (iv). All the above show that there is a strong knockdown-recovery relationship that

can be further quantified through this quick diagnostic, especially towards the separation of weak from strong resistance.

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CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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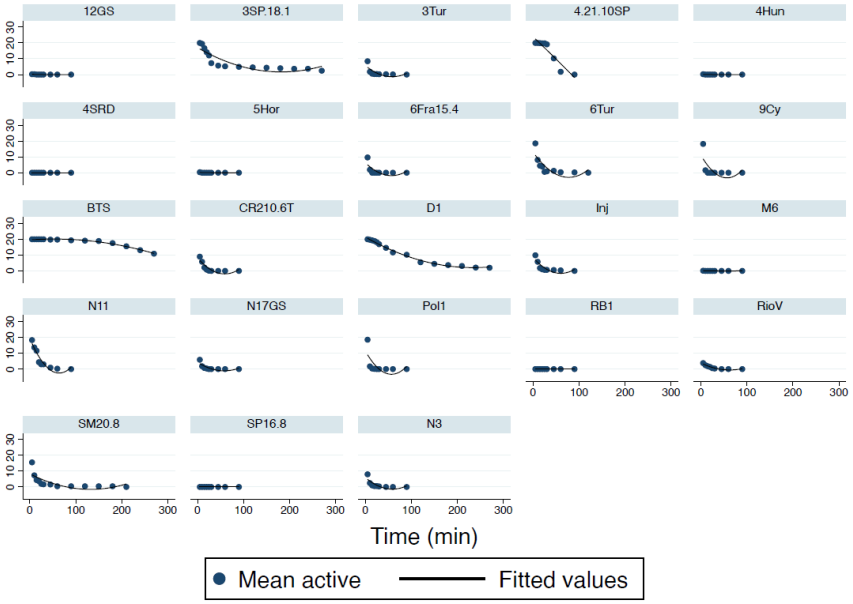
Table 1: Populations of *T. castaneum* that were evaluated for phosphine resistance, with their code, origin and collection date.

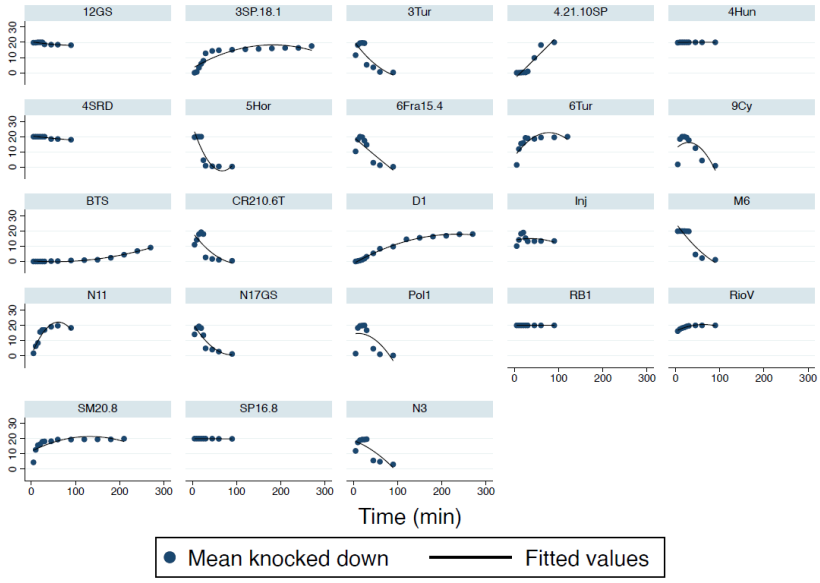
Code	Country	Month/Year of collection
12GS	Serbia	1/2017
3SP18.1	Spain	1/2017
3Tur	Turkey	9/2016
4.21.10SP	Spain	10/2016
4Hun	Hungary	7/2016
4SRD	Spain	1/2017
5Hor	Hungary	10/2016
6Fra15.4	France	4/2017
6Tur	Turkey	9/2016
9Cy	Cyprus	12/2016
BTS	Serbia	5/2016
CR210.6T	Czech Republic	6/2017
D1	Bangladesh	6/2016
Inj	Serbia	5/2016
M6	Brazil	4/2016
N11	Serbia	12/2016
N17GS	Serbia	12/2016
Pol1	Poland	9/2016
RB1	Brazil	12/2017
RioV	Brazil	5/2016
SM20.8	Spain	8/2016
SP16.8	Spain	8/2016
N3	Serbia	12/2016

Figure 1: Relationship between active (A), knocked down (B) and immobilized (C) adults and time after exposure to 3000 ppm at different intervals. Second degree polynomial fitting is shown for reference.

A



B



C

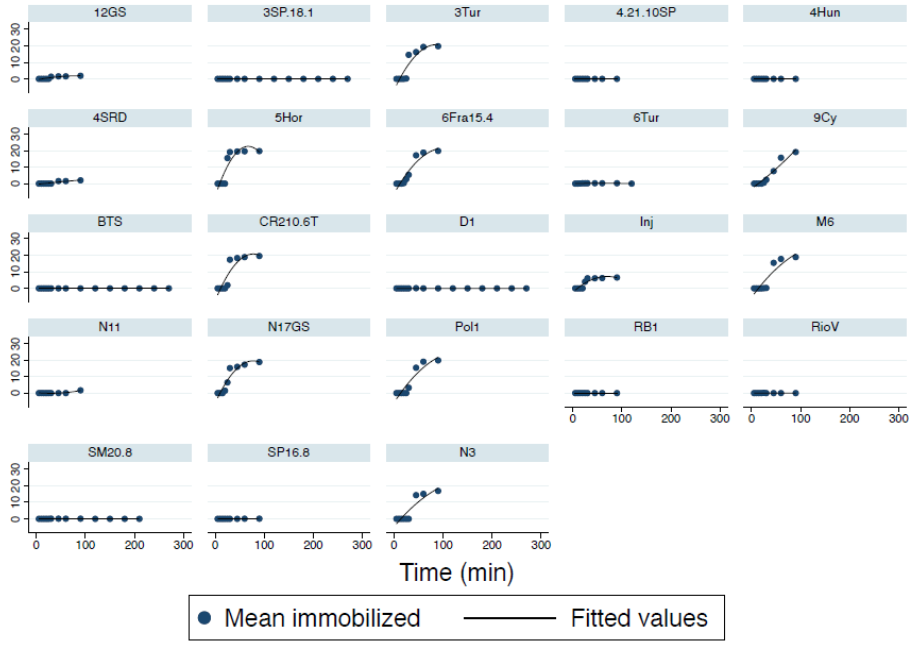
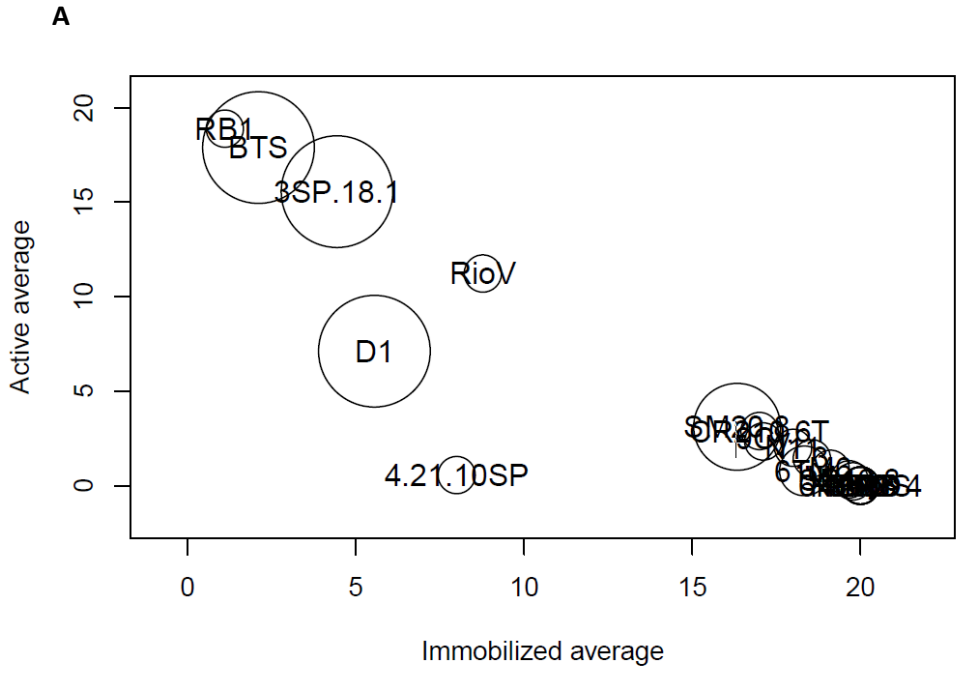
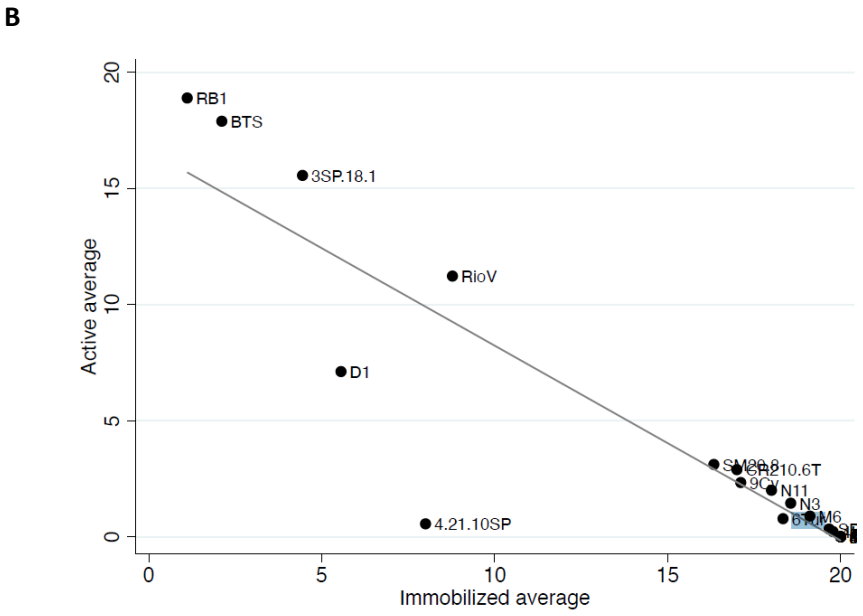
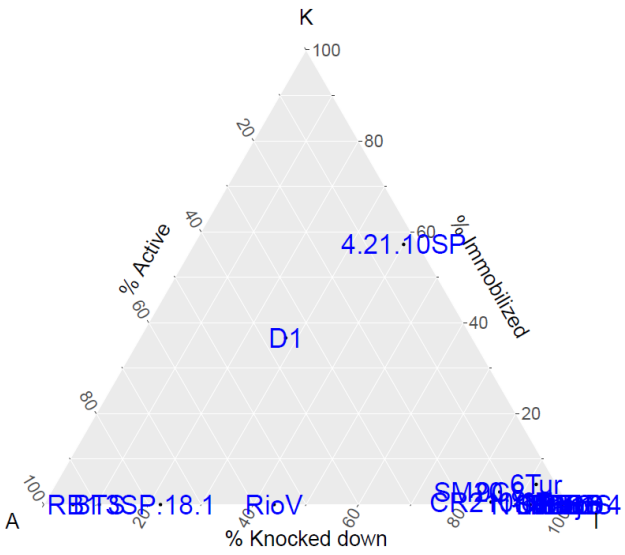


Figure 2: (A) Relationship between active time and immobilized time of adults 7 days post exposure. The maximum time is represented by the bubble size around the population identity. (B) Linear fit between time active vs time immobilized and (C) The ternary plot represents three-dimensional complementary data (active + knocked down + immobilized = 20), with identities of each population after 7 days post exposure, expressed as percentages.





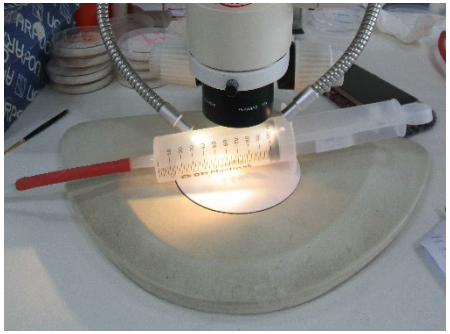
C



Graphical abstract

Phosphine resistance was evaluated using a modification of Detia Degesch Phosphine Tolerance Test Kit (DDPTTK)

Different populations of T. castaneum were used



Active, knocked down and immobilized insects were recorded from 5 to 270 minutes and after 7 days post exposure

Results

- High level of phosphine resistance was recorded
- Knocked down leads easier to mortality than to recovery
- No survivals were recorded after 7 days post exposure for 8 out of 23 population tested