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Resistance to the Fumigant Phosphine and Its Management in Insect Pests of Stored Products: A Global Perspective

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Abstract

Development of resistance in major grain insect pest species to the key fumigant phosphine (hydrogen phosphide) across the globe has put the viability and sustainability of phosphine in jeopardy. The resistance problem has been aggravated over the past two decades, due mostly to the lack of suitable alternatives matching the major attributes of phosphine, including its low price, ease of application, proven effectiveness against a broad pest spectrum, compatibility with most storage conditions, and international acceptance as a residue-free treatment. In this review, we critically analyze the published literature in the area of phosphine resistance with special emphasis on the methods available for detection of resistance, the genetic basis of resistance development, key management strategies, and research gaps that need to be addressed.

INTRODUCTION

Disinfestation of stored products, including grain, dried fruits, nuts, cocoa, coffee, and other processed food products, using fumigants has been in practice for more than a century. Phosphine (hydrogen phosphide, PH_3) is an essential fumigant used globally to disinfest stored products, thus maintaining the integrity of food, and to facilitate international trade through the provision of pest- and residue-free commodities. The key to phosphine's success and popularity in stored product pest control over several decades includes its ease of application in different storage structures, effectiveness against major pest species, relatively low cost, and acceptance by markets and regulatory authorities as a residue-free treatment (76).

Over the past three decades, with the gradual phase-out and restricted use of the fumigant methyl bromide due to its ozone-depleting nature (121), there has been an overreliance on phosphine by industry. During this time, phosphine resistance has increased in frequency, distribution, and strength in multiple pest insect species, a situation that is aggravated further by the lack of suitable alternatives. Despite the severity of the problem, there is no consistency in how resistance is diagnosed and characterized, and there is a limited understanding of the science behind phosphine toxicity and no serious or concerted efforts to develop rational, science-based management strategies. Published reviews on phosphine resistance are useful but narrowly focused (16, 22, 35, 76, 117). Our objective in this review is to provide a comprehensive synthesis of the literature on all aspects of phosphine resistance in insect pests and its management for durable stored products. This review can be a useful resource for global research and practitioner communities engaged in combating this problem and identifying needs for future work.

A UNIQUE FUMIGANT

Phosphine has had a nearly 100-year of history of use as a fumigant after it was first described chemically by Philippe Gengembre in 1783 as a product of heating elemental phosphorus in a solution of potassium carbonate (40). While phosphine has been used to disinfest bulk commodities, it did not become commonplace until the 1960s, when the so-called liquid fumigants were lost due to regulatory actions (118). The solid phosphine salt formulations that generate phosphine gas upon reaction with water under warm temperatures, such as aluminum phosphide and magnesium phosphide, are those most commonly used for durable commodities.

Several positive attributes make phosphine a unique and valuable tool for stored product protection. Firstly, it has the lowest cost among available fumigants for stored products industry, and it is relatively easy to apply. Secondly, the commercial formulations as solid tablets, blankets, and sachets or as a cylindered gas facilitate its application across many types of storage structure, whether a silo, a bag stack, pad storage, a bunker, railcars, a shipping container, or a large-bulk shipload (20). Another key attribute is the acceptance of phosphine as a residue-free treatment by international markets. Phosphine can disperse rapidly through the commodity due to its similar density to air. Moreover, phosphine breaks down quickly after fumigation, making it atmospherically safer than methyl bromide (20). These combined characteristics have made phosphine a unique fumigant unmatched by alternatives such as sulfuryl fluoride, carbonyl sulfide, propylene oxide, ethyl formate, and hydrogen cyanide (76).

DEVELOPMENT OF RESISTANCE IN KEY PESTS

Geographic Distribution, Trends, and Frequencies

Phosphine resistance, as with resistance to any insecticide, is a genetically controlled and heritable trait allowing the carriers to survive a dose of phosphine that would normally kill conspecifics

lacking that trait. Scientifically based determination of phosphine resistance in storage pests began with the development of a diagnostic dose assay by a special working group of the United Nations Food and Agriculture Organization (FAO) (36). Utilizing this so-called FAO test, Champ & Dyte (21) undertook a global survey that provided a starting point for the historical accounting of phosphine resistance in storage pests. The FAO test determines the presence of resistance in a population based on the survival of adult beetles following exposure to a discriminating dose for that species under standard laboratory conditions. The global survey included samples for eight beetle species from over 250 locations in approximately 60 countries representing the six major continental regions of the world (see **Supplemental Table 1**). Six of the eight species in the survey were reported with resistance, whereas no resistance was found in *Sitophilus zeamais* Motschulsky and *Oryzaephilus mercator* (Fauvel). Species with the highest incidence of resistance were *Rhyzopertha dominica* (F.), with 23% of 94 locations, and *Tribolium confusum* Jacquelin du Val, with 30% of 92 locations.

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In the 40 years since the global survey (21), there have been reports of control failures from several countries that suggested that higher or stronger levels of resistance had developed in some species (25, 62, 73, 94). Research over this period in Australia led to the characterization of two phenotypes for two levels of resistance, weak and strong, and the FAO method was modified with higher doses to detect these phenotypes (29). The basis of resistance was elaborated using classical and molecular genetics that identified genes at two separate loci conferring either weak or strong resistant phenotypes (56, 107, 109). **Supplemental Table 2** summarizes the first detections of strong resistance in key pest species.

Australia has a long history of resistance monitoring at a national level that records data in a central database called the Australian Grain Insect Resistance Database (AGIRD) (35). Analysis of 20 years of AGIRD data recently suggested that, although the frequency of the common weak resistance has increased significantly over this period and is currently between 60–80% (depending on species), the frequency of strong resistance has remained below 10% for *R. dominica* (F.) (27), *Sitophilus oryzae* (L.) (45), and *Tribolium castaneum* (Herbst) (77). Also in Australia, Nayak et al. (73) reported a very high frequency of strong resistance incidences in *Cryptolestes ferrugineus* (Stephens) and the psocid *Liposcelis bostrychophila* Badonnel (74).

Research in Asia reported strongly resistant populations of *R. dominica* from Bangladesh (69), India (94), China (100), and the Philippines (1); *S. oryzae* from India (94), Vietnam (80), and China (62); *C. ferrugineus* from China (62); *Liposcelis entomophila* (Enderlein) from Indonesia (86) and China (17); *L. bostrychophila* from India (93); and *Trogoderma granarium* Everts from Pakistan (5).

In North America, surveys in the state of Oklahoma show a significant increase in the frequency of resistance in both *T. castaneum* and *R. dominica* over two decades (83). Subsequent work in North America highlighted increases in frequency and strength of phosphine resistance in common stored product pests *R. dominica* (3), *T. castaneum* (19, 39), *C. ferrugineus* (61), *Plodia interpunctella* (Hübner) (39), and *Lasioderma serricorne* (F.) (104). Resistance frequencies for some populations of these species were in the higher range of 80–100%.

Reports of phosphine resistance in South America include that of Lorini et al. (63), who surveyed *R. dominica* populations in Brazil and diagnosed 14 of the 19 samples as strongly resistant. Other studies from Brazil reported that a weak resistance frequency of 90% was detected in *S. zeamais* (88), and a 100% weak resistance frequency was detected in field populations of *T. castaneum*, *R. dominica*, and *Oryzaephilus surinamensis* (L.) (87).

Very limited resistance data are available from Europe. There was a recent report on resistance in *T. castaneum* from Turkey (60) and reported resistance in *Sitophilus granarius* L., *R. dominica*, and *T. castaneum* from the Czech Republic (8–10). More recently, Greek researchers undertook

an extensive survey in grain storages across Greece for the first time and detected resistance in populations of several species (4).

Only two reports of phosphine resistance are available from Africa; one was conducted in Morocco, where 50 out of 51 *S. oryzae* populations were detected with resistance (14), and the other was a case of strong resistance in *T. granarium* in Burkina Faso (12).

Characterizing the Strength of Resistance

The FAO test is useful for detecting and monitoring resistance frequencies but is limited for determining the presence of strong resistance in populations with positive FAO results (28). Research in the past 20 years has developed methods to characterize the strength of resistance and conducted experiments with homozygous resistant populations, as critical parts of developing strategies to manage resistance (25, 26, 63, 72, 73).

The characterization of phosphine resistance as being weak or strong includes the following. Once a population is diagnosed as resistant, a group of adults from this population is exposed to a range of low to high concentrations of phosphine, and the dose-mortality data from respective concentrations are then subjected to probit regression analyses (38). A resistance factor or ratio (RR) is then calculated by dividing the LC_{50} (the lethal concentration estimated to kill 50% of the tested insects) for a resistant population by the LC_{50} for a reference susceptible population. Generally, populations with a substantial number of heterozygous individuals, which may be phenotypically susceptible or weakly resistant, lead to poor fit to the probit model and thus need further selection to achieve homozygosity for that level of resistance. Work characterizing weak and strong phosphine resistance phenotypes finds RR50 values for weakly resistant populations to be less than 50, while strongly resistant populations may have RR50 values from 50 to over 1,000 depending on the type of dose-mortality trial conducted (refer to **Supplemental Table 2**). Any individuals surviving higher concentrations are reared and exposed to a series of higher concentrations each generation for at least 6–8 generations before homozygosity for resistance is achieved (25). This homozygous population represents the typical field selection and resistance development scenario for that particular species. This population is then used for development of practical fumigation strategies in the laboratory prior to their implementation in the field (26, 63, 72, 73).

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Emerging Problems

Phosphine has potential as an alternative to methyl bromide for quarantine treatment, but the broad geographic occurrence of phosphine resistance in many pests raises the concern that phosphine may be a poor quarantine fumigant. Although most stored product insects are distributed in most countries worldwide, infested shipments are not tolerated in international trade and require either commercial or regulatory applications of phosphine at the time of shipment. One pest with strictly enforced quarantine status is *T. granarium*, the Khapra beetle. Although established in much of Asia and Africa, it is not established in most of Europe, the Americas, and Oceania, whose countries practice diligent inspections and routine methyl bromide fumigations (6). Information on the resistance status of quarantine pests should be considered a priority.

Insects from common pest species are well-known to grain inspectors as they evaluate grain in commerce (44). The less obvious pests that may go undetected during inspections include several psocid species, in which strong levels of phosphine resistance are well documented (72, 75). Other nontarget pests like mites or tiny moths could have evolved resistance to phosphine and may pose cross-border biosecurity threats.

GENETIC BASIS OF RESISTANCE

Inheritance and Number of Genes Involved

Genetic analysis of weak resistance in several species suggested that a single major recessive gene was responsible for the trait (13, 25, 31). Strong resistance in *R. dominica* was caused by variants of two major genes (25, 66, 67, 106), which was subsequently also found to be the case in *T. castaneum* (49), *S. oryzae* (80, 81), and *C. ferrugineus* (50). An analysis of strong resistance in *R. dominica* collected from widely separated regions across eastern Australia provided the first evidence that independent resistance outbreaks were due to variants of the same two genes (67).

A molecular genetic study in *R. dominica* mapped the two resistance genes, *rph1* and *rph2* (resistance to phosphine 1 and 2) (106). Each gene independently conferred weak resistance when homozygous for a resistance allele (20 times for *rph1* and 12.5 times for *rph2*), but when both genes were homozygous for resistance in a single insect, they interacted synergistically to produce the strong resistance trait (250 times). The advent of high-throughput genome sequencing facilitated the identification of the *rph2* gene and its variants in *R. dominica* (109) and *T. castaneum* (51) and, later, in *S. oryzae* (80). This gene encodes a key respiratory enzyme, dihydrolipoamide dehydrogenase (DLD), that involves aerobic energy metabolism (115). Analyses of insects from Australia, the United States, India, Vietnam, Turkey, and China revealed that in every insect that was examined, strong resistance was invariably associated with homozygosity of resistance alleles of the *rph2* gene (23, 50, 59, 60, 67, 80, 109).

The *rph1* gene has also been identified through various gene-mapping techniques carried out on multiple pest species (51, 107). Through the process of elimination, a single gene that is associated with resistance in each of *R. dominica*, *T. castaneum*, *S. oryzae*, and *C. ferrugineus* was identified. This gene was then identified to be the resistance factor *rph1*. The *rph1* gene encodes a fatty acid desaturase (FADS).

Mode of Action

The following mechanism of phosphine toxicity proposed by Schlipalius et al. (109) relies on the fact that the DLD enzyme (rph2) generates reactive oxygen species (ROS) as a by-product of its normal role in aerobic respiration (115). The FADS enzyme (rph1) generates desaturated fatty acids, which are targets of ROS (107). Exposure to phosphine exacerbates ROS production, which causes oxidative damage to the fatty acids of cellular membranes. Thus, the synergistic interaction between rph1 and rph2 derives from the normal function of FADS (rph1), which sensitizes animals to ROS (107), and the ability of DLD (rph2) to generate large amounts of ROS (115), which is exacerbated by phosphine exposure (22). When insects are homozygous for resistance alleles of rph1, cellular membranes are less vulnerable to ROS. When insects are homozygous for resistance alleles of rph2, less ROS is produced. Individuals homozygous for resistance alleles of both genes not only produce less ROS, but also are less vulnerable to the ROS that is produced, resulting in extremely high levels of phosphine resistance.

This model is consistent with the observation that oxidative stress is a key mediator of phosphine toxicity (22), and that the toxicity of phosphine requires aerobic respiration, in which DLD is a key participant (109). Genetic disruptions (127) and conditions that alter the rate of aerobic respiration, such as hypoxia (55) or exposure to mitochondrial activators (122), demonstrate a strong positive correlation between aerobic respiration and phosphine toxicity. Furthermore, antioxidants that detoxify ROS or protect against oxidative damage protect against phosphine toxicity (48).

DIAGNOSTIC METHODS

FAO Tests

The recommended diagnostic dose to assess phosphine resistance is a concentration of gas equal to the dose estimated to achieve $LD_{99.9}$ for beetles from a susceptible population when exposed for 20 h at 25°C, followed by a confirmatory end-point mortality 14 days after fumigation (36). Use of adult beetles, rather than beetles at other life stages, was recommended for ease and consistency in light of challenges with isolating immature life stages for some grain insects. Detailed studies are needed to describe variation in tolerance among different life stages of the resistant population (123). Examples of diagnostic doses for phosphine resistance in common storage pests are as little as 20 ppm for *R. dominica* and up to 50 ppm for *S. granarius*.

With the characterization of two levels of resistance, the FAO method was modified, and two discriminating doses (concentration and exposure period) were established to diagnose weak and strong levels of resistance. For example, the discriminating doses to determine weak and strong resistance in *R. dominica* are 20 ppm for 20 h and 180 ppm for 48 h, respectively (27); 30 ppm and 180 ppm for 20 h for *S. oryzae* (45); and 20 ppm and 180 ppm for 20 h for *T. castaneum* (77).

Rapid Knockdown Tests

A drawback with mortality bioassays such as the FAO method is the 14-day period needed to reach a conclusion on the resistance status of a population. To address this, there has been continuous effort to develop same-day, rapid, or quick assays based on determining the exposure time of a known concentration of phosphine to knock susceptible adult insects down in a test sample. Basically, the knockdown criterion proposed in these studies implies the inability of insects to move in a coordinated manner. Based on this principle, rapid resistance diagnostic tests were developed for several stored product pests, including *T. castaneum* (19, 99, 114), *R. dominica* (2, 29), *S. oryzae* (11, 70), *L. serricorne* (47), *O. surinamensis*, and *S. granarius* (114). The commercial field test kit developed by Steuerwald et al. (114) (referred to as the Degesch test kit) uses 3,000 ppm against adult insects and classifies them as either active or knocked down. Based on the kit instructions, the presence of active insects after 8, 11, 12, and 13 min indicate the presence of resistance to phosphine in *T. castaneum*, *O. surinamensis*, *S. granarius*, and *C. ferrugineus*, respectively.

Nayak et al. (73) developed a quick test for *C. ferrugineus*, which allows for a diagnosis of weak and strong resistance within 5 h of exposing the adult insects to 1,440 ppm of phosphine. This was followed by another development of a quick test that enables the determination of weak and strong resistance in *S. oryzae* within 3 h at 1,440 ppm and within 1.5 h at 3,600 ppm (79). Using the conditions of the Degesh test kit, at 3,000 ppm, a knockdown time of 180 minutes was determined to be suitable to classify strong resistance in *T. castaneum* (18), and at least 200 minutes was determined to be suitable for strongly resistant *R. dominica* populations (2).

Ideally, research on quick tests such as those described above should enable researchers to diagnose resistance of field samples on the same day as their arrival in the laboratory. Same-day advice to industry facilitates timely implementation of necessary management tactics. Improvements to the Degesch test kit, including safe generation and disposal of test gas, with protocols modified to determine both weak and strong levels of resistance, could allow for broader use by industry.

Molecular Diagnostics

The identification of the strong resistance gene, *rph2*, made the development of diagnostic molecular assays possible (23, 56, 59, 60, 108, 109). Strong resistance from *rph2* requires the simultaneous

presence of homozygous resistance variants of both *rph1* and *rph2* genes, but in developing the molecular resistance assay, we simply ignored *rph1* and assumed that resistance variants of *rph1* were always present. This was possible because resistance variants of *rph1* were already very common throughout Australia and the world at the time that the *rph2* gene was identified (105).

The enzyme encoded by the *rph2* gene is so important to aerobic energy metabolism (109) that only seven changes to the amino acid sequence of DLD (59) have been found (**Supplemental Table 3**) that can confer resistance to phosphine while preserving function of the enzyme sufficiently to allow the insects to survive in the field. The strategy developed for screening large numbers of field-collected insects for all resistance variants relies on polymerase chain reaction (PCR) amplification of each exon of the *rph2* gene using tagged primers. The amplified DNA from all of the insects is then pooled prior to identification of resistance variants using high-throughput DNA sequencing. The tags on the primers allow each sequence variant to be attributed to the insect from which it originated for thousands of insects at one time (108).

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ECONOMIC IMPACT OF PHOSPHINE RESISTANCE

Biosecurity regulations for commodities during trade have become increasingly stringent in view of public demand for clean, uninfested products. For example, Australia has adopted a nil tolerance policy for live insects in grain destined for international markets, and this principle is also being implemented for the domestic market (43). Quarantine regulations are imposed on key insect pests, including *T. granarium* and *Prostephanus truncatus* (Horn), to restrict their movements (71, 120). Failure to achieve complete control of pests during trade can prove to be very costly, as reflected in the recent rejection of an US$84 million load of US soft red wheat due to the detection of live insects (37). Although Taylor (116) recorded phosphine resistance in *T. granarium*, there has been no published information on any fumigation protocols available to control this pest with phosphine, which is an ongoing challenge for the industry. Failure of phosphine to control resistant pests will require alternative treatments that can be very expensive. For example, in Australia, compared to the cheapest option of phosphine (US$0.25/ton), the use of an alternative such as sulfuryl fluoride can be very costly (US$3.00/ton) (42).

ECOLOGICAL IMPLICATIONS OF PHOSPHINE RESISTANCE

Fitness

Researchers have used a range of methodological approaches to investigate potential fitness costs associated with phosphine resistance, with some researchers demonstrating fitness costs and others finding none. One approach is to see if physiological or ecological parameters are correlated with resistance ratios across a range of resistant populations. Respiration rate, development, and reproduction were negatively correlated with resistance ratio in several species (89, 113). Other researchers have used the population cage approach in which resistant and susceptible strains are hybridized; the resulting populations were reared in the absence of phosphine for multiple generations while being monitored for changes in resistance level (using bioassays) or resistance gene frequency (using molecular screening). Based on monitoring for changes in resistance levels, there appear to be no fitness costs associated with the *rph1* or *rph2* resistance genes in several species (31, 32, 49, 105). However, in one case where both resistance level and gene frequencies were monitored in an experiment on *T. castaneum*, while there was no clear trend with resistance level (49), there was an increasing trend with the *rph1* gene and a decreasing trend with the *rph2* gene (51). In another study on *T. castaneum*, being strongly resistant appeared to have a

negative impact on flight and walking behavior (64). From research completed to date, it appears that phosphine resistance may come with fitness costs, but these may occur only under specific circumstances.

Movement, Population Structure, and Gene Flow

The movement of grain by humans provides a means of passive movement of stored grain pests in farm machinery, trains, and other means of transport and has the potential to contribute to the spread of resistance (82, 85, 111). Active movement through flight in some of these pests occurs not only where grain is stored, but also in the landscape more broadly, potentially contributing to the spread of resistance (33, 34, 46, 92, 102, 103). Several recent population genetics studies provided indirect evidence of movement by demonstrating the extent of gene flow and population structure over large geographic scales (68, 102, 103, 119). Another study used paternity analysis to show that the majority of *T. castaneum* and *R. dominica* females trapped in flight had mated with more than one male (91). Therefore, it is possible that resistant females surviving a fumigation selection event may be carrying both resistant and susceptible variants of *rph1* and *rph2*, acquired through mating before or after the selection event. Conversely, susceptible females in the broader environment may be carrying resistant variants of *rph1* and *rph2* despite never being exposed to phosphine. The existence of polyandry in *T. castaneum* and *R. dominica*, and potentially other species, needs to be considered when developing models of phosphine resistance.

Distribution and Spread of Resistance Gene Variants in the Field

Molecular screening to determine the spatiotemporal patterns of resistance gene frequencies can enhance our understanding of the development and spread of phosphine resistance. To date, however, few studies using this approach have been published. Although alleles of two major genes (*rph1* and *rph2*) are known to confer phosphine resistance, molecular diagnostics for genotyping insects with phosphine resistant variants are known only for the *rph2* gene; these diagnostics give insight into population genetics for resistance in the studies discussed below.

Samples of *R. dominica* were collected from farms in Queensland, Australia, and screened for the K142E variant of the *rph2* gene (56). Despite all of the samples being classified as weakly resistant based on phenotype testing, beetles that were carriers of the *rph2* resistance variant were detected in samples from both years, and the majority of these carriers were heterozygotes. Two of the 10 samples from 2006 and all six samples from 2011 contained the *rph2* resistance variants. The estimated resistance variant frequency was 4–6% for 2003 and 3–26% for 2011. Many of the farms sampled in this study were certified organic and phosphine had not been used for 10–15 years, suggesting that the presence of this allele on these farms was the result of migration from areas where phosphine was in use. Research with *T. castaneum* from Kansas in the United States also found that resistance alleles at the *rph2* locus were in several populations that did not have very many beetles with the strong resistance phenotype (see **Supplemental Figure 1**).

Supplemental Material >

In another Australian study, samples of *R. dominica* were collected from 59 sites across eastern and southern Australia in a 12-month period during 2008–2009 and screened for multiple variants of the *rph2* allele (108) (see **Supplemental Figure 2**). Despite 7% of the samples expressing the strong resistance trait, 49% of the samples contained beetles that had at least one copy of an *rph2* resistance variant. Four variants were detected, with the most common being P49S, followed by the K142E, G135S, and N506H variants. The P49S and K142E variants had large overlapping distributions, and several samples contained both of these variants. In samples in which any of the variants were detected, the frequency of carriers of the *rph2* allele was 4–79%. Significantly,

the frequency of *rph2* carriers was higher in samples collected from bunkers (i.e., large grain piles covered with plastic sheeting).

The proline to serine amino acid substitution in *R. dominica* (P49S) and the corresponding change in *T. castaneum* (P45S) deserve special mention. This resistance variant dominates the pest populations analyzed in surveys carried out in India (59) and Turkey (60) and is also the most common and most widely distributed variant found in eastern Australia (108); it is also found in the United States (23). The persistence of this variant is likely due to two factors, the strength of the resistance trait conferred by the variant and the preservation of reproductive fitness despite the change to an essential enzyme of energy metabolism.

These studies show the potential for using molecular screening to investigate the distribution and spread of resistance gene variants in the field, as well as how little is known in this regard. Regardless of the molecular approach used, studies with strong spatial or temporal elements (or both) are needed.

MANAGING RESISTANCE

Monitoring of Resistance

A critical step in the management of resistance is the anticipation of it before control measures actually fail. For example, since the 1990s, Australia has had almost three decades of a nationally coordinated phosphine resistance monitoring program that has helped the grain industry by providing early warning of resistance developments and temporal trends and geographic spread (27, 31, 32, 35, 45, 77). Although Australia has benefited from several decades of almost continuous monitoring, periodic monitoring is valuable. In the United States, for example, recent resistance surveys have established that strongly resistant populations are present in grain-growing states (3, 19, 83), a significant change since the 1990s (126) and a clear warning to the industry. While resistance monitoring has traditionally been based on bioassays, recent developments open up the possibility of using molecular diagnostics to screen for the presence of resistance variants in pest populations across the grain value chain (108).

Consideration needs to be given to how resistance monitoring is to be done. Recent published studies show that weak resistant populations can be common in many storages (3, 31, 32, 59, 108). In places like these, therefore, monitoring using tests that detect weak and strong resistance, or even strong resistance only, would be more valuable. In eastern Australia, for example, most or all populations tested will be characterized as resistant, but most of these will contain weakly resistant phenotypes (31, 32, 108).

Reducing Selection

It is a well-established fact that phosphine resistance develops in a pest population mainly due to failure to maintain the recommended concentration within the storage enclosure, resulting in selection for resistance, given that resistance genotypes are present (15, 24). Several factors can contribute to inadequate fumigation, including leaky storage structures, underdosing, and fumigation temperatures that are lower than that recommended for phosphine fumigation (20, 24, 76, 90, 110). Repeated fumigation in an attempt to control surviving populations in leaky storage is a typical example of selection for resistance (41).

Collins (24) outlines a comprehensive account of strategies to reduce the selection pressure for phosphine resistance. These include ensuring the airtightness of storage structures, limiting the number of repeat fumigations on the same batch of commodity, minimizing the application of

phosphine through rotation with other treatments including grain protectant or another fumigant, reducing the number of insects exposed to selection by regular storage hygiene, and eradicating resistant populations prior to their spread. In Australia, a regulatory standard requires that silos be pressure tested to confirm airtightness prior to their use (24, 41). Moreover, a well-coordinated national grain storage extension team plays a critical role in facilitating the adoption of best pest and resistance management practices, including on-site demonstrations to growers of pressure testing of silos (41).

Optimizing Phosphine Fumigation Regimes to Control Resistant Populations

Several biological and non-biological factors affect phosphine efficacy and should be considered in research aimed at optimizing phosphine fumigations. An important biological factor affecting phosphine efficacy is insect developmental stage. Eggs and pupae of resistant insects tend to be more tolerant to phosphine than larvae or adults (123). In addition, delayed egg hatching after exposure to phosphine has been reported for *T. castaneum* (95), *R. dominica* (97), *L. bostrychophila* (74), and *C. ferrugineus* (98). It is critical, therefore, that experiments aimed at improving phosphine efficacy against resistant insects take into consideration such biological factors. For example, specific developmental stages can be tested (57, 58, 72), or populations containing all developmental life stages can be tested (26, 30), to ensure that meaningful recommendations can be made.

Two important non-biological factors affecting phosphine efficacy are concentration and exposure time, both of which can be manipulated to maximize phosphine's toxicity against insects. Studies show that increasing either concentration or time will increase efficacy against resistant insects, but the effects are unequal, with time usually being more important than concentration (26, 30, 57). Temperature is also important, with phosphine efficacy proven to be higher at higher temperatures (57, 72).

Laboratory studies can provide important information leading to improved phosphine fumigations of immediate relevance to industry. In this regard, the Australian registration label for cylindered phosphine is largely based on data generated from laboratory experiments on phosphine-resistant populations exposed to constant concentrations (26, 72). In other cases, translating laboratory data into practical guidelines is a challenge. For example, fumigations that use aluminum phosphide as the source of phosphine typically have phosphine concentrations that vary greatly over the course of the fumigation. Despite the need for commercial-scale fumigation experiments, few such studies have been published. Rajendran & Muralidharan (96) fumigated bag stacks containing paddy rice and provided detailed data on phosphine concentration over time and efficacy data against resident infestations, including resistant *R. dominica*. Wang et al. (124) fumigated bag stacks containing paddy rice and established efficacy data against resistant *R. dominica* and *Cryptolestes* species in cages. Ridley et al. (101) fumigated silo bags containing sorghum, monitored phosphine concentration over the fumigation period, and presented efficacy data against resistant *R. dominica* populations in cages. More field studies like these are needed to provide data to underpin recommendations about optimizing phosphine fumigations.

Strategic Use of Alternative Fumigants

Several fumigants have been evaluated as alternatives to phosphine. Sulfuryl fluoride (SO_2F_2) is registered in some countries for use on stored products, and recent laboratory studies have found no evidence of cross-resistance to sulfuryl fluoride in phosphine resistant *R. dominica*, *T. castaneum*, *S. oryzae*, or *C. ferrugineus* (52, 53). Field studies have further confirmed the potential for using this fumigant as an alternative to phosphine. Opit et al. (84) fumigated small silos resulting in high

levels of control of infestations of phosphine-resistant *R. dominica* and *T. castaneum*. Nayak et al. (78) carried out a series of large-scale fumigations of bunker storages and achieved complete control of caged populations of strongly phosphine-resistant *R. dominica*, *C. ferrugineus*, *T. castaneum*, and *S. oryzae*. Other fumigant treatments have shown potential in laboratory experiments to control phosphine-resistant insects in a range of species; these treatments include ozone (112), chlorine dioxide (125), and combinations of phosphine with carbon dioxide (7, 65) or sulfuryl fluoride (54).

Practical Implementation of an Integrated Strategy

Successful management of phosphine resistance will require an integrated approach, including resistance monitoring, optimizing phosphine, fumigations, and strategic use of alternative fumigants. An example is the response to the development of strongly resistant *C. ferrugineus* in eastern and southern Australia. Following control failures at large grain handling facilities that used registered fumigation protocols, researchers confirmed the existence of a new strong resistance in this species (50, 73). New fumigation protocols were developed for these types of facilities based on new research data (57). Affected grain companies developed an eradication strategy in collaboration with researchers involving regular monitoring, strategic use of sulfuryl fluoride only in case of failure of phosphine, isolation of grain with resistant pests, treatment with registered contact insecticides, and adoption of an intensive hygiene program (78).

CONCLUSIONS

This review highlights the growing global problem of the development of phosphine resistance in major stored products pests. This resistance threatens the sustainability of phosphine as the cheapest and most versatile fumigant for disinfestation of stored products. Major progress has been made with the inheritance and biochemistry of resistance. Two major genes are responsible for resistance, with resistance expressed as two major phenotypes (i.e., weak or strong). Historically, the FAO diagnostic test and its variations have underpinned resistance surveys, but the development of same-day knockdown tests offer the possibility of faster testing, and molecular diagnostics allow for rapid and accurate screening for resistance genes. Quantification of the effects of phosphine concentration, exposure period, and other variables such as temperature is providing a basis for the development of effective fumigation protocols for resistant populations. Over the past decade, we have gleaned new insights into the ecological implications of phosphine resistance from field studies on dispersal, gene flow, and polyandry. There are ongoing attempts in many countries to manage strong levels of resistance in major pests that are seriously compromising the effectiveness of currently registered rates of phosphine. Management options include the early detection of strong levels of resistance through monitoring, characterization of resistance, development of improved phosphine fumigation protocols, and use of alternative treatments. However, several areas need attention from future research that will help in extending the usefulness of this unique fumigant into the foreseeable future.

FUTURE ISSUES

1. Modification of the FAO method and development of a research-based, field-validated, and universally agreed-upon phosphine resistance detection method are required for detection of both weak and strong levels of resistance in major pest species of stored products.

2. It is important to complete the development and validation of the molecular resistance diagnostic platform to detect all possible genetic variants responsible for strong resistance in major pest species.
3. A coordinated global survey for strong resistance in major pest species using agreed-upon bioassay and molecular diagnostic methods is overdue.
4. Development and field validation of effective phosphine fumigation protocols for resistant populations of major stored products pests, including the quarantine pest *T. granarium*, are priorities.
5. Development and field validation of alternative fumigants are imperative to manage phosphine-resistant pest species.
6. Development of an integrated phosphine resistance management decision-making system and its field validation is critical. Key aspects of this system may include early warnings on development of resistance and accurate determination of the strength of resistance, timely interventions and use of suitable alternatives to control resistance, and the evaluation of the success of these interventions.

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