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Genetic Control Tactic Against Fruit Flies (Diptera: Tephritidae) Insect to Escape Destruction of Perishable Horticulture Crops

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Abstract

The goal of this paper is to show that the precise genetic components that determine competence in a broad sense or fitness in the narrower ecological sense are extremely economical and more important for pest fruit flies control. The true fruit flies (Tephritidae) are the question of quarantine and control efforts as a consequence of their extensive agricultural impact and large range of expansions worldwide. Among these flies, the pests that infest fruits, squashes and other cucurbitaceous plants result in heavy crop damage and subsequent monetary losses. Of these species, flies mainly belonging to the genus Bactrocera are *Bactrocera zonata* (Saunders), *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) having a higher detections frequency, broader host range, and pose a greater economic loss to agriculture than other fruit fly species. The increased frequency of travel and trade in recent years has resulted in increased detection of non-native and invasive pests. The sterile insect technique (SIT) is an environment friendly and species-specific method of pest control based on the release of large numbers of sterilized insects. The increased introduction rate of these pests, the development of resistance by the pests to conventional chemical control measures, and the commitment to develop and use alternative integrated pest management (IPM) measures have made the effective use of sterile insect technique. Some of the techniques developed in molecular genetics that may be useful in autocidal control programs for fly insect pests include, molecular biology and transgenesis, genetically modified release of insects carrying a dominant lethal, and genetic sexing technique. Developing and implementing an integrated pest management program is highly requested to minimize the use of chemical pesticides to overcome these above mentioned problems and produce fruit fly-free and residue-free fruit and vegetable to facilitate compliance with standards required for export markets.

Keywords

Bioinvasion, Autocidal Control, Insect Transgenics, Fruit Files, Irradiation

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1. Introduction

Fruit and vegetable productions in various regions are affected by a number of fruit fly species (Diptera: Tephritidae). Pest fruit flies cause direct yield losses and can restrict fruit and vegetable exports due to quarantine restraints inflicted by introducing countries to limit the spread of these pests. True fruit flies are found in nearly all habitats with suitable plant life. Their cosmopolitan distributions, broad larval host range and substantial economic impacts have placed tephritids among the world's most notorious agricultural pests (McPheron and Steck, 1996). Tephritids in the genus Bactrocera are of particular concern throughout much of Asia and Australia, where these constitute a significant threat to agricultural resources

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(Kinnear et al., 1998; Kim et al., 1999). Perhaps these species economically are more important; the threat of their migration to uninfected areas is the rationale for nearly universal quarantine regulations against agricultural products from infested areas. If we could control or eliminate the threat from fruit flies to the point where direct losses are negligible and quarantine regulations are no longer necessary, we can expect improved productivity and increased agricultural diversification in infested areas. The long term goal of fruit fly control is to enhance long-term viability and competitiveness of agricultural systems in areas currently at risk (Stephen and Susan, 1993).

2. Fruit Fly Species of Economic Concern

The genera Anastrepha, Bactrocera, Ceratitis, Dacus, Rhagoletis and Toxotrypana of fruit fly species are serious pests of agriculture throughout the world and represent a threat to the agriculture and ecology of the certain states. Because of their wide host ranges, their abilities to become established or to become more widespread, their potential economic impacts and their potential ecological impacts (both direct and indirect), the species in those genera have been subjected to strict quarantines and comprehensive control programs. The plant pests selected for inclusion in this environmental impact statement are the species of tephritid fruit flies mainly belonging to the genus Bactrocera are *Bactrocera zonata* (Saunders), *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett). These species are selected based upon their on-going threat to agriculture and the basic research already conducted to develop genetically engineered strains that can be adapted for use in control programs (Sarwar, 2014 a; 2014 b).

3. Threat of Fruit Flies Pest Species

The fruit flies are a major and most destructive pest of agriculture throughout many parts of the world. Because of their wide host range of fruits and vegetables species, and potential for damage, the fruit flies represent a serious threat to horticulture. The female has a pointed slender ovipositor to deposit eggs under the skin of host fruit. The female fly attacks ripening fruit, piercing the soft skin and laying eggs in the puncture. The eggs are minute cylinders like laid in batches and eggs hatch into larvae (maggots) which feed inside the fruit pulp. The maggots (larvae) are creamy-white, legless and may attain a length of 10 millimeters inside host fruit. In many states, the fruit flies could attack apples, grapes, kumquats, loquats, nectarines, peppers, persimmons and several nuts. These are very serious pests of various fruits, particularly guava, ber, citrus, mango and papayas. Fruit flies also attack a wide variety of other fruits and vegetables, and their maggots have been found in several kinds of produces in many parts of the world. A great number of other crops threatened by the introduction of these pests include pears, plums, berries, cherries, peaches, apricots, figs, avocados and tomatoes. Based on the pest situation, this paper therefore aims to offers the more developed effective and accurate detection and genetic control methods for fruit fly pests in the entire global regions (Sarwar et al., 2013; 2014 a; 2014 b; 2014 c).

4. The Proposed Action against Fruit Flies

Current control techniques for fruit flies rely on spraying of chemical insecticides. These methods may have limited in scope and can vary in their applicability to different fruit fly species. The actions being considered in the preferred alternative of control for fruit fly pests provide programs with new and potentially more cost-effective methods. These methods can resulting in substantial reductions in operating costs and improved efficiency for the on-going fruit fly control programs. These methods would also benefit by making more effective use of the limited personnel and limited space available at insect rearing facilities for these programs needed by the general public, industry, academic, regulatory, and public interest groups. There are several laboratory and confined field studies to test the efficacy of certain genetic engineering applications that could provide benefits to these programs.

4.1. Mass Trapping of Fruit Flies

Mass trapping reduces fruit fly populations by attracting fruit flies to traps where these become stuck or are exposed to a minute amount of pesticide, and die before they have the opportunity to mate. The fruit flies are attracted to a bait at the traps (conventional fruit fly traps, sticky panels, fibre board squares, wicks or bait spots on telephone poles or roadside trees), where these become stuck with a sticky substance or are killed with a minute amount of pesticide (naled or malathion). Mass trapping has potential for many species of fruit flies but is not effective for all species. There are some limits to the use of mass trapping, the approach is costly and labor-intensive, may require placement and servicing of 1,000 or more panels or traps per square mile within the infestation area. Effectiveness is reduced if these are dislodged and inadvertently destroyed by the public, livestock or pets. Finally, traps are believed most effective when new infestations are detected and integrated controls are used, but are believed ineffective for large populations where the fruit flies have mated prior to being trapped by the panels, and the lures (natural and synthetic) have not proven equally effective on all species of fruit flies (Harold, 2001).

4.2. The Sterile Insect Technique

Control of fruit flies currently relies overwhelmingly on the use of chemical insecticides, and because of the high economic and environmental costs of chemical control, together with the appearance of insecticide-resistant populations, so, there is an urgent need for improved control methods. The sterile insect technique (SIT) presents an alternative, environmentally friendly and species-specific method of population control. This is only non-chemical method currently available for control of a pest through the release of large numbers of sterilized flies. The sterile males mate find wild type females, mate with them, and thereby sterilize them, so the theory goes forth. There is evidence that this technique works in some cases, and it is being widely used for fruit flies. In a small factory, the flies are bred of a few millions in a week at peak time; the files are sterilized at a nearby nuclear facility, and then released as pupae. A problem with this technique in its most basic form is that the large scale breeding produces large numbers of females as well as males. Extra irradiation doses needs to be given to ensure that fertile females are not released, and the evidence also indicates that the sterile males are less likely to look for wild females if many sterile ones are available at the point of release. With millions of flies being produced, sexing on an individual scale is impossible. However there are ways of producing so-called genetic sexing strains. In Medfly the Y chromosome, which contains the genes causing the fly to be male, has been joined on to another chromosome containing a particular wild type gene. A mutant form of this gene causes lethality in double dose, but in males, if the mutant gene is protected by the wild type gene, flies are normal. This system needs to be 'conditional', otherwise all females would die. In Medfly the lethal gene is temperature sensitive, so that a burst of high temperature at the early larval stage kills the females (David, 2008).

4.3. Genetic Sexing Technique

In many applications of autocidal control, it would be efficient to separate the males and females before release. Possible reasons for such separation are to avoid assortative mating and to avoid any increase in the size of the feral population. The fruit flies are mass reared for release in several areas. Part of the present fly mass-rearing process involves production of only males through a temperature sensitive lethal (TSL) strain of the fruit fly in SIT programs. Females die at a temperature above 29°C. By putting the fruit fly eggs in a water bath at the threshold temperature, the females are killed and only the males survive to later be irradiated and released to mate with wild-type females, which then produce no offspring. This has been achieved through selective breeding over many years and is not a result of genetic engineering. The development of the TSL strain has greatly assisted in increasing the fly production at these facilities and the sterile insects needed in emergency programs. One comment encourages use of transgenic sexing systems, referred to as temperature sensitive lethal (TSL) systems for sterile insect technique. However, currently available transgenic genetic sexing systems, which kill females, actually operate on the same molecular biochemistry as the Release of Insects carrying a dominant lethal (gene or genetic system); a system in which insects are engineered to carry dominant lethal mutations in their gametes to induce sterility by genetics rather than achieving sterility by exposure to radiation. The introduction of genetic sexing strains (GSS) opens up the possibility of using the SIT for suppression as opposed to eradication and different radiation strategies can be considered (Robinson, 2002). Sex-separation is a simple consequence of withdrawing the food additive from the final factory generation and no sterilization step is required.

4.4. Release of Insects Carrying a Dominant Lethal (RIDL) System

Withdrawal of the repressor chemical from the diet in the last factory generation would be lethal to all the females, resulting in a single-sex male population. If these males are then sterilized by irradiation and released, there would have combined conventional SIT with an efficient genetic sexing mechanism. This mechanism alone would be a major advance but in fact this single-sex male population can be released as a control agent without sterilization. This system is called Release of insects carrying a dominant lethal (RIDL) as the males are not, strictly speaking, sterile. The comments mainly focuses on the use of the transgene autocidal system versus radiation for SIT programs and suggests that genetic sexing strains could further benefit SIT programs. However, filter-rearing systems, which are closely managed, are required for temperature sensitive lethal (TSL) sexing strains. These strains are currently the best sexing technology for the Medfly only, because reversion to wild-type insects commonly occurs.

Although SIT has been very successful against other tephritid pests, the key problems included altered diurnal mating rhythms of the laboratory-reared insects, resulting in asynchronous mating activity between the wild and released sterile populations, and low competitiveness of the radiation-sterilized mass-reared flies. Consequently, the production of competitive, male-only release cohorts is considered an essential prerequisite for successful olive fly SIT. Results developed a set of conditional female-lethal strains of olive fly (named release of insects carrying a dominant lethal-RIDL), providing highly penetrant female-specific lethality, dominant fluorescent marking and genetic sterility. The males of the lead strain, are strongly sexually competitive with wild olive flies, display synchronous mating activity with wild females and induce appropriate refractoriness to wild female re-mating. Furthermore, through a large proof of principle experiment, that weekly releases of males into stable populations of caged wild-type olive fly could cause rapid population collapse and eventual eradication. The observed mating characteristics strongly suggest that an approach based on the release of males will overcome the key difficulties encountered in previous olive fly SIT attempts. Although field confirmation is required, the proof of principle suppression and elimination of caged wild-type olive fly populations through male releases provides evidence for the female-specific RIDL approach as a viable method of olive fly control (Alphey and Andreasen, 2002; Ant et al., 2012).

4.5. Genetically Modified (GM) Fruit Flies

Producing Genetically Modified (GM) flies insect with the aim for open release into the environment is an improvement to the sterile insect technique, which has been applied successfully to a number of other pest species. However, in comparison with SIT, a major problem with the use of GM agricultural pests is that rather than being sterile, the female offspring of the matings between GM males and wild females die mostly at the late larval or pupal stage i.e., towards the end of the larval (maggot) life stage. Because the larval stage is when flies damage the fruit, it is unlikely that this approach can actually prevent much of the damage to the crop, even if it successfully suppresses the wild population. The fact that the female offspring of GM insects survive to the late larval or early pupal stage (in the absence of tetracycline) is unintended, so that no eggs are laid or no larvae are produced, this would avoid the damage that the GM larvae will cause to the crop. A second problem is the efficacy of this approach to reduce olive fly populations, and the "release ratio" of GM males to wild males that may be required. This means that GM males may need to vastly outnumber wild males if these are to mate successfully. Such large numbers suggest that GM insects may be no more competitive with wild males than irradiated insects are.

An important difference between the sterile insect technique using irradiated insects and the release of genetically modified insects is that radiation-induced sterility involves multiple sites of damage to insects' DNA, whereas the RIDL system relies on a specific genetic modification. This means that, unlike with irradiated insects, GM insects which survive and breed successfully i.e., that overcome the genetic 'late-lethality' mechanism, could evolve rapidly during mass production. If this happens, the lethality effect could rapidly disappear as resistance develops in production facilities or in the field. Another potential mechanism for resistance is that wild females may become unreceptive to mate with released males. This implies that, even if population suppression is effective, resistance could develop relatively rapidly. Mass breeding of GM insects will also result in loss of fitness over time (due to inbreeding, known as the "colony effect"). Loss of fitness means that fewer males will mate with wild females and effectiveness will be reduced. In the use of irradiated SIT, new wild insects can be added to the colony prior to irradiation in order to increase the fitness (Alphey, 2002; Morrison et al., 2010).

4.6. Molecular Biology and Transgenesis

Recent advances in molecular biology and transgenesis research have renewed interest in genetically based control methods because these advances may remove some major technical problems that have constrained effective genetic manipulation of pest species. Population genetic analyses suggest that transgenic manipulations may enable development of strains that would be 10 to over 100 times more efficient than strains developed by classical methods. Some of the proposed molecular approaches to genetic control involve modifications of classical approaches such as conditional lethality, whereas others are novel. Experience from the classical era of genetic control research indicates that the population structure and population dynamics of the target population will determine which, if any, genetic control approaches would be appropriate for addressing a specific problem. As such, there continues to be a need for on-going communication between scientists who are developing strains and those who study the native pest populations (Gould and Schliekelman, 2004).

5. Integration into Pest Management Programs

The research of scientists integrates using the tools of entomology, plant physiology, genetics and molecular biology to find methods of improving and lowering the costs of crop production. The sterile insect release method and other autocidal control techniques are completely compatible with other types of insect control that might be used in IPM programs. In fact, scientists have always insisted that autocidal control must be integrated with other measures in order to be used in the most effective way. The autocidal control technologies are most efficient and economical when pest populations are already at low levels. One reason for this is that these techniques generally involve release of laboratory (or factory) reared insects. Thus, the numbers of insects that can be reared will determine the size of the area of release. If less native insects are in the release zone, then a higher release ratio will be attained or a larger release area can be covered. Any insect control measure that will reduce population densities either before or during the application of an autocidal control program will enhance the effectiveness of both procedures. For example, use of biocontrol organisms is generally most effective at high pest densities, but lose efficiency as pest densities decrease. Thus, a sterile insect release program would be a natural adjunct to any type of biological control program. This same reasoning applies to the use of insecticides to reduce high pest populations and the use of pheromones in confusion programs. Cultural control methods, which are generally used to reduce pest populations during their most vulnerable stages during the year, are completely compatible with the use of autocidal control programs because the latter programs would generally be used during the time of high reproductive potential of the target species. It is hard to think of an integrated pest management program that could not be enhanced by the addition of an autocidal control technique (Knipling, 1985; Bartlett, 1990).

Each of the above cited alternative action has the potential for adverse environmental consequences. Those consequences are related principally to the use or the non-use of control methods. The integrated program alternative would offer the greatest flexibility for responding to fruit fly pests and would have the least indirect (and long-range) adverse impacts, but it could have greater direct adverse impacts. The preferred alternative, an integrated program, offers the greatest flexibility in responding to fruit fly pest outbreaks. With an integrated program, nonchemical and chemical controls would be available to program managers, based upon the exigencies of the outbreak. Nonchemical methods, including sterile insect technique, can be used in coordination with chemical methods in emergency eradication programs or may be used as the principal method in some suppression programs. The preferred alternative, thus, accommodates eradication or suppression programs, and allows the use of nonchemical controls, chemical controls or both (Sarwar, 2004; 2012; 2013; Shah et al., 2014; Sarwar et al., 2015).

6. Conclusion

Fruit flies belonging to Family Tephritidae are considered the most important insect pests that cause enormous damage for a wide host range of horticultures all over the world. The most important conclusion coming from the study is that there is a core population of such fly pests. The cooperative programs involve the use of IPM to eradicate fruit fly. In addition, the methods used to eradicate and control infestations of these fruit flies have been developed and proven to be effective. Specifically, the integrated program allows the option for use of the following methods, chemical control, sterile insect technique, physical control, cultural control, male annihilation by use of selective male insect attractants (lures) combined with insecticides or placed in insect traps, and regulatory control. The chemical treatments involved in this integrated approach include soil drenches, foliar sprays and fruit fly male annihilation spot treatments. The certification option that has been developed involves the use of an area-wide SIT program combined with underlying fruit fly surveillance and monitoring trapping array. A proposal of an international program (a broad strategy) among global countries should be prepared to respond to the threat of these invasive pest species. Such program must include a range of alternative options including exclusion, detection and prevention controls. To achieve these goals, an understanding and extensive studies on the biology and ecology of any insect pest is considered the corner stone to establish effective control measures that might enable us to win the battle against such pests.

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