RESEARCH PROJECT IN

MICROBIAL BIOTECHNOLOGY

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1. Research project

Baculovirus-based insecticides interactions with lepidopteran hosts and host food plants: useful information for applying correct IPM programs in Crop Protection systems

1. RESEARCH PROJECT

Background

The application of chemical insecticides has been the principal method for the control of agricultural insect pests over the past 60 years. However, the availability of effective compounds has become increasingly limited by the development of resistance in phytophagous pest populations (Roush and Tabashnik, 1990; Rodríguez et al., 2010; Torres-Vila et al., 2002) and the removal of a number of older broad-spectrum compounds from the marketplace (European Parliament Regulation (EC) 1107/2009). At the same time, farmers are attempting to reduce the use of chemical insecticides to meet the demands generated by increasing public concerns regarding the presence of insecticide residues in food (Chandler et al., 2008). Additionally their incompatibility with natural enemies and pollinators markedly limits the use of broad-spectrum phytosanitary products, being urgently required the development of highly selective control agents to control lepidopteran pests without adverse impacts on beneficial insect populations. Suitable control of insect pest complexes can usually be achieved by the development of systems based on integrated pest management (IPM) that emphasize ecologically or technologically-based strategies of control (Pérez-Hedo et al., 2017; Thomas, 1999). Such a concept aims to avoid placing undue emphasis on a single technology (chemical control, biological control, transgenic plants, etc.), but rather combines elements of each type of technology in the most compatible manner to maintain pest populations below economic injury threshold levels (Cook et al., 2007; Kogan, 1998). In addition since 2014, IPM is mandatory in any crop system in Spain (Royal Decree 1311/2012, which incorporates European Directive 2009/128/EEC).

Biological control based on predator and parasitoid measures offer an effective solution for the management of certain pests, but often fail to provide effective control of important Noctuidae lepidopterous (Fuentes et al., 2017a). Therefore, microbial insecticides become necessary components of IPM systems that can contribute to overcoming the limited range of chemical insecticide options available in certain crops, and provide the basis for policies aimed at reducing both farmer dependence on chemical pesticides and the presence of xenobiotic residues in food (Hossain et al., 2017; Tatchel, 1997). Insect baculoviruses (BVs) have a proven track record as effective and highly selective biological insecticides for the control of a range of lepidopteran pest species (Moscardi 1999; Szewczyk et al., 2009). Numerous studies have demonstrated the existence of genotypic variation between and within BV isolates (Arrizubieta et al., 2013, 2015a; Barrera et al., 2011, 2013; Bernal et al., 2013a,b; Simón et al., 2004). Genotypically distinct isolates of the same BV species or even genotypes from a single isolate have shown to vary in phenotypic traits fundamental in pathogen fitness; such as transmission, speed of kill, yield and persistence, or adaptation to host plant (Arrizubieta et al., 2015a; Barrera et al., 2013; Bernal et al., 2013b; Cory and Hoover, 2006; Hodgson et al., 2002; Simón et al., 2004). The isolation of individual genotypic variants has facilitated the genotypic characterization of wild-type populations and has allowed the evaluation of their relative biological activity contributing to our understanding of their diversity and evolution. Infections with experimental mixtures of different genotypes of a given viral species or different viral species have revealed antagonistic or synergistic effects in the phenotypic traits resulting from these interactions. In some instances, the mixture of viral genotypes enhances the efficiency of the virus as a biological insecticide (Arrizubieta et al., 2015a; Bernal et al., 2013b; López-Ferber et al., 2003; Simón et al., 2005), but contrasting results have also been reported in other virus-host systems (Barrera et al., 2013; Muñoz and Caballero 2000). Therefore, when developing BVs as biopesticides, single genotypes or, more commonly, specific genotypic mixtures, with improved characteristics, are selected. Therefore, genomic and biological characterization of the viral populations is strongly recommended before the release of new BV insecticides.

Following this approach, the Microbial Bioinsecticides Group has developed a technique for the production of virus occlusion bodies that occlude virions comprising genomes of different baculovirus species or genotypes from the same viral species that can be used to combat different insect pest (PCT/P2013/069678). This technique has allowed the development of several BV bioinsectides that included a mixture of genotypes in a certain proportion that increases its transmissibility. For example, a binary mixture of HaNPV genotypic variants has improved insecticidal characteristics to control cotton bollworm than the pure genotypes or the wild type isolate (Arrizubieta et al., 2015a). Similarly, the co-occluded mixture that included three genotypes of ChchNPV at 4:3:1 proportion killed larvae 33 h faster than wild-type population (Bernal et al., 2013b). These two mixtures and the process to obtain them have been patented (WO2015/197900A1 and PCT/EP2014/056762, respectively) and transfer to a life-science company. This underlines the importance of understanding not only the diversity of isolates but also the interactions between them, to select those with well defined activity profiles for specific biocontrol programs.

The presence of this variation raises some interesting questions, particularly with regard to the maintenance of BV diversity and it effects on BV-host interactions, as these phenotypic differences may alter BV-host population dynamics that clearly influence the transmissibility and efficacy of those genotypes or mixtures as biological control agents (Goulson et al., 2002). Through the isolation of individual genotypes the effects of host-ecology on the fitness of co-infecting parasites have been investigated. Insects often feed on several species of food plant, and food plant species can have a major impact on the phenotypic traits and persistence of insect parasites (Goulson et al., 2002; Hoover et al., 1998). Moreover, selectable plants traits have been shown to enhance entomopathogens efficacy, as entomopathogens may impose selection pressure on plant traits that benefit both plant and entomopathogen fitness (Shikano, 2017). Further, red (Shikano et al., 2017a). The fact that plant species can affect pathogen fitness and that there is genetic and phenotypic variation in insect pathogen populations, make it not unreasonable to assume that plant species influence parasite population genetic structure, as well as host insect. Therefore, differential selection of several BV genotypes, mediated by host food plant species or any other ecological category, may promote the persistence and stability of BV populations.

Host food plant mediates the differential selection of BV genotypes, indirectly by influencing the development of insect host (larvae that grow larger by feeding on a specific host food plant might produce greater yields), or directly when larvae ingested secondary chemicals produced by the plant that affect BV fitness (Cory and Hoover, 2006; Goulson et al., 2002; Shikano, 2017). Firstly, any chemicals that alter the pH of the midgut affect the dissolution rate of BVs. The chemical ecology of the host-food plant interaction may therefore provide sufficiently different gut and cell conditions in the host to influence selection pressures on genes for virus pathogenicity and within-host replication. Secondly, differences between food plants in acidity and the production of secondary chemicals, such as peroxidase and hydrolysable tannins, influenced BV fitness. Tannins in particular are known to bind to virus coat proteins and also restrict access to midgut epithelial cells by reducing the size of pores in the peritrophic membrane (Shikano et al., 2017b). Moreover, plant phytochemicals have been shown to accelerate the onset of sloughing off the midgut cells, one of the resistance routes to BV infection (Hoover et al., 2000). Since phenolic oxidation is an important component of plant resistance against insects, the effectiveness of BVs in controlling lepidopteran populations may be adversely affected by host food plant varieties that presented high content of host-plant resistance substances against noctuid larvae (Felton et al., 1987; Hoover et al., 2000; Shikano, 2017). However, plants among and within species invest differently in a myriad of chemical and physical defences. Therefore, among the different plant varieties some of them would be able to maintain both high resistance against an insect pest and high efficacy of a BV insecticide. Alternatively, BV genotypes might differ in within-host growth rate, OB degradation rates, and transmission parameters, leading to a complex array of phenotypes.

Objectives

Plants have an important role in the evolution of insect-pathogen relationships, and so, a tritrophic perspective should be evaluated into the study of insects and their pathogen, even more when developing these pathogens as bioinsecticides. The overall objective of this project is to understand the interactions of BV-based insecticides with the key biological and chemical components of IPM programs, with the aim of optimizing the use of these biopesticides in effective and sustainable IPM programs. Moreover, in this project we take investigation of tritrophic interactions involving BVs as a stage further and ask whether host plant can exert differential effects in naturally coexisting parasite genotypes, using BV genotypes, their lepidopteran hosts and host food plants as a model system. To this end a series of studies d**e**scribed below (Research lines) will be performed in laboratory and field conditions. These all investigation lines might bridges an important gap between the ecology of host-pathogen interactions and the population genetic structure of pathogen populations, with the aim of developing more effective BV-based insecticides to implant more effective IPM programs.

Research lines

1. Development of BV based bioinsecticides

1.1. Genetic diversity of BV isolates. The Microbial Bioinsecticides group of the Public University of Navarre has a collection of wild-type isolates of differences BV species from different geographical regions. Additionally, future prospections in different crops will increase this collection. All theses isolates will be characterized by DNA analysys (RFLP and agarose electrophoresis) to determine the genetic identity of each isolate. The restriction patterns of these isolates will allow determining the viral species and its genetic diversity, and it is also reference information to detect possible changes in the genetic compositions of these viruses.

1.2. Selection of BV isolates with high insecticidal characteristics. Genetically different isolates will also be phenotypically characterized. The relative insecticidal activity of each of these isolates will be evaluated in a preliminary assay by a toxicity test using the droplet feeding method (Bernal et al., 2013a). The isolates with greater activity in the preliminary test will be evaluated in fine bioassays to specify the dose-mortality relationship and lethal time in second and fourth instar larvae. The values of median lethal concentration (LC_{50}) and mean lethal time (TL_{50}) will be calculated using a logit or probit analysis.

1.3. Cloning of the pure genotypes present in wild-type isolates. To purify the genotypes present within the different isolates, different biotechnological techniques will be used, such as *in vivo* cloning (Muñoz et al., 1998), *in vitro* cloning (Simón et al., 2004), or bacmid technique (Hitchman, 2002). The different clones will be multiplied in larva, in order to obtain sufficient OBs to make the molecular characterization (REN analysis). The genotypes with different REN profiles will be selected for subsequent assays.

1.4. Identification of the pure genotypes present within selected isolates isolate. The selected genotypes will be sequenced, and by sequencing analysis (blast) specific primers will be designed to identify specific genotypes in wild-type isolates. Additionally, complete genome sequencing will also allow determining genomic differences that may be responsible for those genotypic differences.

1.5. Determining the phenotypic characteristics of pure genotypes or mixtures of genotypes. The phenotypic characterization of pure genotypes or mixtures of genotypes will include at least the determination of the mean lethal concentration (LC_{50}), the mean lethal time (TL_{50}) and the number of inclusion bodies (OBs) produced by infected larvae (OBs/larvae).). The LC_{50} and TL_{50} values will be calculated using a logit or probit analysis. The average number of OBs/larvae will be determined by infecting 40-50 freshly molted fourth stage larvae with a concentration of OBs corresponding to the LC_{95} and by counting the OBs produced by each larva with a hemocytometer (Neubauer) in an optical microscope with phase contrast.

2. Plant effects on the efficacy of BVs as biological control agent.

2.1. Plant effects on BV genotypic composition and efficacy as biological insecticide. It is known that plants influence the interactions between herbivores and their natural enemies, including baculoviruses (Ode, 2006; Shikano, 2017). Plants play an important role in insect-baculovirus interactions, because the insects acquire infective virus transmission stages

(OBs) by feeding on virus-contaminated plant tissues. Phenolic oxidation can interference negatively with infections in the gut. However, plants between and within species might react differentially to insect feeding in a variety of chemical and physical defenses, and so, among the different genotypes of a given crop some of them might be able to maintain low insect resistance and high BV efficiency. Additionally, BV genotypes might react also differently to phenolic oxidation. In the present research line we will investigated the trithropic interactions between plants-herbivores-pathogens. For doing that, firstly BV isolates will be challenged to insect host on different host plants through successive passages and dynamics on these population, by genotypic composition variation, will be studied in detail (Simón et al. 2004). Alternatively, larvae of a given insect pest would be reared on different host food plant species or varieties, then exposed to different BV insecticides and measured the effect of the food plant on virus fitness in terms of larval mortality, speed of kill, and yield of OBs. At the same time, the phenolic composition would be estimated in the different plant species or varieties by measuring plant phytochemicals and peroxidase activity (Felton et al., 1987; Hoover et al., 2000). Finally, the effects on insect development would be measured by determining different parameters related with host development and reproduction (larval and pupal weight, adult emergence, fecundity and fertility) and with susceptibility to a superinfection (Cabodevilla et al., 2011). This all information would be of special interest to select the most suitable genotype or mixture to control insect pests in a given crop system.

2.2. Determining the genes involved in adaptation of BVs to plant-phytophagous ecology. Comparative genomic studies can be used to identify viral genes favored under selection that are involved in host range, pathogenicity and virulence, or plant-phytofagous-pathogen interactions (Simón et al. 2008a, 2008b, 2011, 2012a, 2012b). Thus, a complete genome sequence analysis will be performed to identify genes responsible for the phenotypic characteristics of individual genotypes and tritrophic interactions, as well as those genes undergoing positive selection. Thereafter, bacmid-based homologous recombination systems will be employed to study the effects of gene deletion or substitution on the insecticidal properties of these viruses. To this end genes that play major roles in defining insecticidal characteristics and those responsible of a better adaptation to a given plant-host system will be identified and studied in recombinant and naturally occurring genotypes.

3 Elements for decision making in IPM programs

3.1. Evaluation of the efficacy of the BV insecticide. Several parameters will be of special interest to be determined in order to do a correct implementation of these BV insecticides in IPM programs. In this point the efficacy, persistence and rate of acquisition will be determined as these parameters are useful for the UT estimation, and they would give us an idea of the need to formulate the virus.

3.1.1. Efficacy of the BV insecticide. A series of experiments will be carried out in field to evaluate the efficacy of the BV insecticide, including as negative control a water without virus treatment. As positive control the normally applied insecticides will be used. The experimental design will be a randomized block design. To evaluate the efficacy of the treatment, samples of larvae of the central plants of each plot will be collected and mortality will be recorded (Arrizubieta et al., 2015b; Fuentes et al., 2017a; Simón et al., 2015).

3.1.2. Persistence of the virus on the leaf surface. From the edge plants, samples of leaves will be collected and the insecticidal activity will be determined by a bioassay method (Arrizubieta et al., 2015b).

3.1.3. Rate of acquisition of infection. The same experimental design and the same treatments as in activity 5.1.1 will be used. Each plant will be previously infested with larvae and at different intervals after application, larvae will be collected and raised in the laboratory to determine the percentage of virus infection (Simón et al., 2015).

3.2. Estimation of economic injury (EIT) and treatment thresholds (TT) as the basis for decision making. The economic injury threshold can be calculated based on a simple model namely: C = V×I×D×K×EIT where C is the cost of treatment (product + applications costs), V is the market value of the harvested product, IxD represent the reduction in yield due to insect damage, which is calculated experimentally, and K is the efficacy of the treatment. Specifically in this case is necessary to determine defoliation-induced crop losses (Fuentes et al., 2017b). For each virus, K will be calculated from the field studies on BV insecticides efficacy applied to a given crop. Finally, the TT is calculated based on the EIT, but taking into account that the speed of kill of BV insecticides varies according to the stage of the insect larva that is present on the crop at the moment of treatment (Bernal et al., 2014), and the dose of virus applied and consumed by the pest (Simón et al., 2015).

3.3. Integrating BV insecticides with other control agents, especially with natural enemies, and with biorational products used in IPM systems.

3.3.1. *Integration with natural enemies of the target pest*. The combined use of biopesticides and insect natural enemies (predators and parasitoid wasps) that occur naturally or are specifically introduced for pest control purposes has proved to be of recognized value across a range of IPM systems (Cook et al., 2007; Gentz et al., 2010). In such situations each type of natural enemy competes for the same population of hosts or prey and direct competitive interactions can lead to changes in the effectiveness of each type of natural enemy. Specifically, the pathogen may make the host unsuitable for use by the other types of enemies or, conversely, predators and parasitoids may greatly reduce the number of progeny virus particles released by each infected pest caterpillar (Cossentine 2009). It is therefore necessary to perform laboratory studies to determine the impact of the BV on parasitoid development in infected hosts, host immune response, premature death of the parasitoid progeny in infected hosts and the discriminatory capacity of parasitoids toward infected hosts. These studies will lead to a series of laboratory-generated predictions that can be tested under field conditions. Similarly, the impact of natural enemies on the susceptibility of parasitized hosts to BV infections, the ability of predators and parasitoids to disperse virus from infected to susceptible hosts and the influence of parasitism on the phenotypic characteristics of the virus, will be evaluated in laboratory studies and subsequently validated in field experiments to generate specific recommendations for the integration of these different types of natural enemies.

3.3.2. *Integration with other biorational pest control products*. Current pest control measures depend heavily on the use of new generation biorational pest control compounds that have a relatively favourable ecotoxicological profile such as imidacloprid, indoxacarb and naturally-derived products such as spinosad, avermectin, neem and *Bacillus thuringiensis* toxins. These products have become widely used in IPM systems due to their selectivity and efficacy (Fuentes et al., 2017b; Rossell et al., 2008). However, excessive reliance on these compounds has already leaded to pest resistance in some lepidoperan populations. Moreover, pest susceptibility to BV insecticides can vary significantly in the presence of residues of other biorational products such as spinosad (Méndez et al. 2002) or *Bacillus thuringiensis* (Liu et al., 2006). It is therefore necessary to determine the possible additive, antagonistic or synergistic interactions that are likely to occur between BV and other phytosanitary products commonly applied for the control of insect pests. As mentioned above, laboratory experiments will be used to develop specific predictions regarding BV interactions with biorational products that can be explicitly tested in field conditions to define important areas of potential incompatibility or enhanced pest control efficacy.

References

Arrizubieta M, Williams T, Caballero P, Simón O, 2013. Selection of a nucleopolyhedrovirus isolate from *Helicoverpa armigera* as the basis for a biological insecticide. Pest Manag Sci 70, 967-976.

Arrizubieta M, Simón O, Williams T, Caballero P, 2015a. A novel binary mixture of *Helicoverpa armigera* single nucleopolyhedorvirus genotypic variants has improved insecticidal characteristics for control of cotton bollworms. Appl Environ Microbiol 81, 3984-3993.

Arrizubieta M, Simón O, Torres-Vila LM, Figueiredo E, Mendiola J, Mexia A, Caballero P, Williams T, 2015b. Insecticidal efficacy and persistence of a co-occluded binary mixture of Helicoverpa armigera nucleopolyhedrovurs (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula. Pest manag Sci 72, 660-670.

Arrizubieta M, Simón O, Williams T, Caballero P, 2016. Determinant factors in the production of a co-occluded binary mixture of Helicoverpa armigera alphabaculvoirus (HearNPV) genotypes with desirable insecticidal characteristics. PLoS ONE 11, e0164486.

Barrera G, Williams T, Villamizar L, Caballero P, Simón O, 2013. Deletion genotypes reduce occlusion body potency but increase occlusion body production in a Colombian Spodoptera frugiperda nucleopolyhedorivurs population. PLoS ONE 8, e77271.

Barrera G, Simón O, Villamizar L, Williams T, Caballero P, 2011. *Spodoptera frugiperda* nucleopolyhedrovirus as a potential biological insecticide: genetic and phenotypic comparison of field isolates from Colombia. Biol Control 58, 113-120.

Bernal A, Williams T, Hernández-Suárez E, Carnero A, Caballero P, Simón O, 2013a. A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: the basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banan crops. Biol Cotrol 67, 101-110.

Bernal A, Simón O, Williams T, Muñoz D, Caballero P, 2013b. A *Chrysodeixis chalcites* single-nucleocapsid nucelopolyhedrovirus population from the Canary Islands is genotypically structured to maximize survival. Appl Envirom Microbiol 79, 7709-7718.

Bernal A, Simón O, Williams T, Caballero P, 2014. Stage-insecticidal characteristics of a nucleopolyhedrovirus isolate from Chrysodeixis chalcites enhanced by optical brighteners. Pest Manag Sci 70, 798-804.

Bernal A, Simón O, Williams T, Muñoz D, Caballero P, 2017. Remarkably efficient production of a highly insecticidal Chrysodeixis chalcites nucleopolyhedrovirus (chchNPV) isolate in its homologous host. Pest Manag Sci. Submitted.

Cabodevilla O, Villar E, Virto C, Murillo R, Williams T, Caballero P, 2011. Intra-and intergenerational persistence of an insect nucleopolyhedrovirus: adverse effects of sublethal disease on host development, reproduction and susceptibility to superinfetcion. Appl Environ Microbiol 77, 2954-2960.

Chandler D, Davidson G, Grant WP, Graeves J, Tatchell GM, 2008. Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Technol 19, 275-283.

Cook SM, Khan ZR, Pickett JA, 2007. The use of push-pull strategies in integrated pest management. Ann Rev Entomol 52, 375-400.

Cory JS, Hoover K, 2006. Plant-mediated effects in insect-pathogen interactions. TREDS Ecol Evol 21, 278-286.

Cossentine JE, 2009. The parasitoid factor in the virulence and spread of lepidopteran baculoviruses. Virol Sin 24, 305-314.

Felton GW, Duffey SS, Vail PV, Kaya HK, Manning J, 1987. Interaction of nuclear polyhedrosis virus with catechols. J Chem Ecol 13, 947-957.

Fuentes EG, Hernández-Suárez E, Simón O, Williams T, Caballero P, 2017a. Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV): natural occurrence and efficacy as a biological insecticide on young banana plants in greenhouse and open-field conditions on the Canary Islands. PLoS ONE 12, e0181384.

Fuentes EG, Hernández-Suárez E, Simón O, Williams T, Caballero P, 2017b. *Chrysodeixis chalcites* a pest of banana crops on the Canary Islands: incidence, economic losses and current control measures. Crop Prot. Accepted.

Gentz MC, Murdoch G, King GF, 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol Control 52, 208-215.

Hodgson DJ, Vanbergen AJ, Hartley SE, Hails RS, Cory JS. 2002. Differential selection of baculovirus genotypes mediated by different species of host food plant. Ecol Letters 5, 512-518.

Hoover K, Yee JL, Schultz CM, Rocke DM, Hammock BD, Duffey SS, 1998. Effects of plant identity and chemical constituents on the efficacy of a baculvoirus against Heliothos virescens. J Chem Ecol 24, 221-252.

Hoover K, Washburn JO, Volkamn LE, 2000. Midgut-based resistance of Heliothis virescens to baculvoirus infection mediated by phytochemicals in cotton. J Ins Physiol 46, 999-1007.

Hossain L, Rahman R, Khan MS, 2017. Alternatives of pesticides, in: Khan M, Rahman M (eds) Pesticide Residue in Foods. Spring, Chapman and Hall, NY and London.

Kogan M, 1998. Integrated pest management: historical perspectives and contemporary developments. Annu Rev Entomol 43, 243-270.

Liu XX, Zhang QW, Li J. 2006. Effects of Cry1Ac toxin of *Bacillus thuringiensis* and nuclear polyhedrosis virus of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) on larval mortality and pupation. J Pest Manag Sci. 62, 729–737.

López-Ferber M, Simón O, Willaims T, Caballeor P. 2003. Defective or effective? Mutualistic interactions between virus genotypes. Proc R Soc Lond B 270, 2249-2255.

Méndez WA, Valle J, Ibarra JE, Cisneros J, Penagos DI, Williams, T. 2002. Spinosad and nucleopolyhedrovirus mixtures for control of *Spodoptera frugiperda* (Lepidóptera: Noctuidae) in maize. Biol Control. 25, 195-206.

Moscardi M, 1999. Assessment of the application of baculvoiruses for the control of Lepidoptera. Ann Rev Entomol 44, 257-289.

Muñoz D, Caballero P, 2000. Persistence and effects of parasitic genotypes in a mixed population of the *Spodoptera exigua* nucleopolyhedrovirus. Biol Control 19, 259-264.

Ode PJ, 2006. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Annu Rev Entomol 51, 163-185.

Pérez-Hedo M, Suay R, Ruocco M, Giorgini M, Poncet C, Urbaneja A, 2017. Resilience and robustness of IPM in protected horticulture in the face of potential invasive pests. Crop Prot 97, 119-127.

Rodríguez MA, Bosch D, Sauphanor B, Avilla J, 2010. Susceptibility to organophosphate insecticides and activity of detoxifying enzymes in Spanish populations of *Cydia pomonella* (Lepidoptera: Tortricidae). J Econ Entomol 103, 482-491.

Roush RT, Tabashnik BE. 1990. Pesticide resistance in arthropods. Springer, Chapman and Hall, NY and London.

Rossell G, Quero C, Coll J, Guerrero A, 2008. Biorational insecticides in pest management. J Pest Sci 33, 103-121.

Shikano I, 2017. Evolutionary ecology of multitrophic interaction between plants, insetc herbivores and entomopathogens. J Chem Ecol 43, 586-598.

Shikano I, McCarthy EM, Elderd BD, Hoover K, 2017a. Plant genotype and induced defences affect the productivity of an insect-killing obligate viral pathogen. J Invertebr Pathol 148, 34-42.

Shikano I, Shumaker KL, Peiffer M, Felton GW, Hoover K, 2017b. Plant-mediated effects on an insect-pathogen interaction vary with intraspecific genetic variation in plant defences. Oecologia 183, 1121-1134.

Simón O, Williams T, López-Ferber M, Caballero P, 2004. Genetic structure of a *Spodoptera frugiperda* nucleopolyhedrovirus population: high prevalence of deletion genotypes. Appl Environ Microbiol 70, 5579-5588.

Simón O, Williams T, López-Ferber M, Caballero P, 2005. Functional importance of deletion mutant genotypes in an onsect nucelopolyhedrovirus population. Appl Environ Microbiol 71, 4254-4262.

Simón O, Williams T, Asensio AC, Ros S, Gaya A, Caballero P, Possee RD, 2008a. Sf29 gene of *Spodoptera frugiperda* multiple nucleopolyhedrovirus is a viral factor that determines the number of virions in occlusion bodies. J Virol 82, 7897-7904.

Simón O, Willaims T, Caballero P, Possee RD, 2008b. Effects of *Acp26* on in vitro and in vivo productivity, pathogenesis and virulence of *Autographa californica* multiple nucleopolyhedrovirus. Virus Res 136, 202-205.

Simón O, Palma L, Beperet I, Muñoz D, López-Ferber M, Caballero P, Williams T, 2011. Sequence comparison between three geographically distinct *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolates: detecting positively selected genes. J Invertebr Pathol 107, 33-42.

Simón O, Palma L, Williams T, López-Ferber M, Caballero P, 2012a. Analysis of a naturally-occurring deletion mutant of *Spodoptera frugiperda* multiple nucleopolyhedrvoris reveals *sf58* as a new *per os* infectivity factor of lepidopteran-infecting baculoviruses. J Invertebr Pathol 109, 117-126.

Simón O, Williams T, López-Ferber M, Caballero P, 2012b. Deletion of *egt* is responsible for the fast-killing henotype of natural deletion genotypes in a spodoptera frugiperda multiple nucleopolyhedrovirus population. J Invertebr Pathol 111, 260-263.

Simón O, Erlandson MA, Frayssinet M, Willaims T, Theilmann DA, Volkoff AN, Caballero P, 2017. *Lacanobia oleracea* nucleopolyhedrovirus (LaolNPV): a new European species of alphabaculovirus with narrow host range. PLoS ONE 12, e0176171.

Szewczyk B, Rabalski L, Krol E, Sihler W, de Souza ML, 2009. Baculovirus pesticides-a safe alternative to chemical protection of plants. J Biopest 2, 209-2016.

Tatchell GM, 1997. Microbial insecticides and IPM: currently and future opportunities for the use of biopesticides, in: Microbial insecticides: novelty or necessity?. Proc. Br. Crop Prot Council Symp 68, 191-200.

Thomas MB, 1999. Ecological approaches and the development of “truly integrated” pest management. Proc Natl Acad Sci USA 96, 5944-5951.

Torres-Vila LM, Rodríguez-Molina MC, Lacasa-Plasencia A, Bielza-Lino P, 2002. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. Crop Prot 21, 1003-1013.

2. KNOWLEDGE TRANSFER

Universities are entrusted with the discovery of knowledge, knowledge that is produced through basic and applied research. The manner in which knowledge is transferred from institutions of higher education to a society varies depending on the type of knowledge that is transferred and the mechanisms that have been established to transfer it. The term knowledge transfer describes today the activities destined to transfer the knowledge, the abilities and the intellectual property of the universities to the companies. It is often referred to as the "third mission" of the university, complementing the traditional functions of education and research. In practice, research and teaching are the most basic forms through which knowledge is created and transferred. Beyond this, the publication and dissemination of research also have a long tradition as a means for the dissemination of new knowledge.

In particular, the transfer of knowledge covers many activities: research contracts, consultation, licensing contracts, young researchers who spend periods in the company that works with university researchers, and new business projects (spin-offs). All this facilitates the flow of knowledge from the academy to the business world. Universities typically employ knowledge transfer activities in education and research, contributing to economic development by transferring academic knowledge to society through a variety of processes.

In terms of transfer activities, patent licenses can be considered as the transfer of traditional technology and involve the exploitation by the companies of the intellectual property generated in the university. On the other hand, the entrepreneurial spirit is divided into two sub-areas: the activities to promote the entrepreneurial spirit, which include training in and support for the entrepreneurial spirit, as well as awareness-raising activities; also the activities of creation of companies that make reference to the installations and resources of the university to foment the new companies (technological parks, incubators). Finally, the collaboration between universities and companies is materialized through the so-called contracts with companies or through public calls as Collaborative Projects, financed by public entities.

Previous research works carried out at the UPNa having the subject of the development of baculovirus-based bioinsecticides have led to the development of 3 patents for biotechnological invention. These technologies have been transferred to the multinational company ArystaLifeScience, which is currently leading the exploitation of them. All this has had a positive impact on UPNa's economy and prestige.

On the other hand, these research works have led to several publications in high-impact articles, as well as articles of dissemination. Similarly, several research contracts and contracts with companies for the development of these viruses have been made. Finally, thanks to these results, several doctoral theses have been developed at the UPNa. Therefore, baculoviruses and their biotechnological aplications are an area of great repercussion with very good results of knowledge transfer.

So, looking at the productivity of similar projects, it is foreseen that thanks to this new project it will be possible to obtain sufficient results to publish several original scientific research articles, as well as scientific dissemination articles and the necessary information to request at least one patent in the Spanish Patent and Trademark Office. Said patent will deal with the obtaining of new bioinsecticides using as selection pressure systems the host insects on the plants that are fed (tritrophic system), including in this case the variable plant never taken into account at the time of developing these viruses as insecticides. As previously mentioned, it is foreseen that the technology of the patents developed in this work will be transferred to different companies based on biotechnology, as has been done on previous occasions.

Some of the results obtained may be published, but others must necessarily be reserved until a date after the date of application of the patent in order not to interfere in obtaining it. The results obtained that can be published will be disseminated using the following mechanisms:

1. Publications in specialized scientific journals of international scope (such as Biological Control, Biocontrol, Journal of Invertebrate Pathology, Journal of Economic Entomology, PlosOne, Agricultural and Forest Entomology, etc.) and in national journals (such as the Bulletin of Plant Health Pest, Phytoma Spain, etc.).

2. Publications in informative magazines of the agrarian sector (such as Phytoma, Navarra Agraria, Agrarian and Livestock Europe, etc.).

3. Contributions to scientific congresses both national (Spanish Society of Applied Entomology, Ecological Agriculture, etc.) and international (Society for Invertebrate Pathology, IOBC Working Group of Insect Pathogens and Insect Parasitic Nematodes, etc.).

4. Press releases, especially from the local press.

5. Participation in discussion forums of new agricultural technologies (eg International Symposium organized by Phytoma Spain).

6. Annual reports for dissemination among the EPOs of the project.

7. Organization of Informative Days, inviting representatives of the sector and other researchers working in the field of phytosanitary protection in the Canary Islands).

8. This project will also contribute to the realization of 1-2 doctoral theses that will be developed within the Doctoral Program of the Department of Agricultural Production of the Public University of Navarra.