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REVIEW
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Management of *Chloridea virescens* (Noctuidae) in blueberries (*Vaccinium corymbosum* L.) to promote sustainable cultivation in Peru: A Review

Manejo de *Chloridea virescens* (Noctuidae) en arándanos (*Vaccinium corymbosum* L.) para promover su cultivo sostenible en Perú: Una revisión

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Abstract

A review of current and specific literature was carried out in order to elaborate a proposal for the management of *Chloridea virescens* in the cultivation of blueberry (*Vaccinium corymbosum* L.), developing strategies in each component of Integrated Pest Management (IPM), including Cultural Control, Ethological Control, Biological Control, and Chemical Control (PBUA and PQUA). Likewise, steps in the genetic improvement for quantitative resistance of the blueberry to this pest (Lepidoptera: Noctuidae) using wild relatives of this crop as a source of resistance genes are proposed.

Keywords: *Quantitative Resistance, Wild Blueberry, Assisted Selection, Genetic Improvement, Chloridea virescens.*

Resumen

Se realizó una revisión de la literatura actual y específica para elaborar una propuesta de manejo de *Chloridea virescens* en el cultivo del arándano (*Vaccinium corymbosum* L.), desarrollando estrategias en cada componente del Manejo Integrado de Plagas (MIP), incluyendo el Control Cultural, el Control Etológico, el Control Biológico y el Control Químico (PBUA y PQUA). Asimismo, se proponen pasos en el mejoramiento genético para la resistencia cuantitativa del arándano a esta plaga (Lepidoptera: Noctuidae) utilizando parientes silvestres de este cultivo como fuente de genes de resistencia.

Palabras clave: Resistencia cuantitativa, arándano silvestre, selección asistida, mejora genética, *Chloridea virescens*.

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INTRODUCTION

The blueberry is a shrub of the Ericaceous family, belonging to the genus *Vaccinium*, which constitutes a group of species widely distributed throughout the northern hemisphere, basically in North America, Central Europe, and Eurasia, also found in South America, and a few in Africa and Madagascar (García et al. 2018). Of the 30 species that make up the genus, only a few are commercially important, with *V. corymbosum* L., which represents approximately 80% of the total cultivated area in the World, standing out, followed in importance by *V. ashei* Reade, with approximately 15%. Among the remaining 5%, *V. angustifolium* Aiton and some hybrids of *V. angustifolium* x *V. corymbosum* stand out (García et al. 2018). These varieties are differentiated by the number of chilling hours they need to break the dormant or resting period of the plant and are grouped as follows: Lowbush blueberry (*V. angustifolium*), Rabbit-eye blueberry (*V. ashei*), and Highbush blueberry (*V. corymbosum*) (Buzeta, 1997). The cultivars of "highbush" are separated into "northern" and "southern" depending on the requirements of chilling hours and winter hardiness (Hancock, 2009).

In Peru, in recent years, *V. corymbosum* has sustained growth in both area and yield, sown mainly on the country's northern coast. Until 2011, Peru had not joined the International Union for the Protection of New Varieties of Plants (UPOV); this conditioned that the initial development was carried out with released varieties: Biloxi, Legacy, Misty, O'Neal, Duke, and Brigitta, among others. Biloxi has been the most successful variety in the low-lying areas, estimated to have covered between 80% and 90% of the productive coast area. In the heights of the mountains, on the other hand, good results have been obtained with Legacy (Febres, 2013). For Sierra Exportadora (2011), the Biloxi, Misty, and Legacy varieties are the ones that best adapt to our country, but we can find other patented varieties with different costs and production management. These varieties, such as Ventura, Millennial, Emerald, Susy Blue, Windsor, Springhigh, Star, and Jewel arrive in Peru through Fall Creek Far & Nursery, an American company that manages, sells, and reproduces the patents obtained by the universities of Florida and Georgia (Gargurevich, 2017).

The international market promotes crop expansion in our country due to the optimal climatic conditions for its development. The main exploitable commercial window is between the end of September and all of October, where there is competition with Argentina and Uruguay. In November and December, Chile is the main competitor with South Africa and Oceania. Given the countries above' unpredictable frost and rain conditions, Peru can take advantage of better international prices (Sierra Exportadora-PCM, 2012; Ministerio de Desarrollo Agrario y Riego [MIDAGRI], 2016). This crop is potential and strategic to be financed by the various competitive funds that Peru has implemented for years such as Innóvate Perú (formerly Fincyt), Fondecyt, and Pnia, among others, just as coffee cultivation has been financed (Dilas-Jiménez & Cernaqué 2017).

According to Agrodata Peru (2020a), in 2019, our country surpassed Chile for the first time and was crowned as the main exporter of blueberries worldwide, with profits of around the US $810 million (the United States, the Netherlands, and China are the main destinations of the prized bluish berries). Thus, blueberries have climbed to second place in the list of Peruvian fruits for export, surpassed by grapes - in the first place - and above avocados. In the 2019 campaign, the USA was the primary destination with US $458 million (57% of the total), followed by the Netherlands with US $179 million (22%), and China rose to US $70 million (9%) (Agrodata Peru, 2020a). Of the five regions of Peru where blueberries are most cultivated, La Libertad represents the significant growing region, concentrating more than 60% of production. The rest is distributed between Ica, Lima, Ancash, and Lambayeque (Agrodata Peru, 2020b).

According to Gestion (2019), in 2015 the national average yield was 9 tons/ha. On the other hand, in 2018 it reached 15 t/ha due to the improvement in the sowing and harvesting technique. La Libertad and Lambayeque regions have the highest yields, which in 2018, reached

17 and 15 t/ha, respectively. On the contrary, the region of Arequipa registers the lowest yields. In the 2019 campaign, Camposol SA led exports with US $173 million, followed by Hortifrut Perú SAC with US $101 million, and Hortifrut Tal with US $68 million (Agrodata Peru, 2020a).

It is expected that in 2020, the blueberry growing areas in Peru will reach approximately 12,500 hectares; For its part, the Pro Arandanos Union calculated that the figure would be around 14,000 hectares next year. "This year we are sure that the blueberry will exceed US$1,000 million in exports and will be the first Peruvian agro-export product," they assure from Pro Arándanos (AgroNegociosPerú, 2020).

Among the critical pests of the blueberry crop, *Chloridea virescens* is one of the most important ones because it causes damage to the shoots, leaves, inflorescences, and fruits, generating reductions in crop yield. In addition, there are strategies for controlling *H. virescens,* such as cultural, ethological, biological, and chemical control; however, these strategies must be used within integrated management.

In recent years, there have been limitations in chemical control since there is a restriction on the use of active ingredients for the cultivation of blueberries, whose production is destined for the international markets of Europe, the United States, and Asia. For this reason, new mechanisms should be used within the chemical control, such as biological products based on *Bacillus thuringiensis,* or other strategies such as genetic improvement of the blueberry crop to reduce the susceptibility to this pest (Contreras-Pérez et al., 2019). On the other hand, there is genetic improvement in this crop, For example, the United States Department of Agriculture (USDA)-Agricultural Research Service (ARS)-National Clonal Germplasm Repository (NCGR) in Corvallis – Oregon, has performed the genotyping of 367 blueberry samples *Vaccinium spp*. detecting 54 cultivars makes up important germplasm for future genetic improvement studies (Bassil et al., 2020).

Based on all those mentioned above, this study aims to identify scientific and technological advances that allow the generation of an Integrated Management proposal of *Chloridea virescens* for the sustainable cultivation of blueberries in Peru.

For this article, an exhaustive review of relevant information related to integrated pest management (IPM) was carried out, emphasizing the cultivation of blueberries, specifically in the management of *Chloridea virescens*. Research articles in journals in the last ten years were preferably consulted. Scientific information databases were consulted, such as SciElo, ScienceDirect, and Springer, among others, and in a complementary way, other sources of information provided information about blueberries in Peru. The keywords used, individually or conjugated, were "blueberry, blueberries, *Chloridea virescens*, integrated management, CRISPR, cry proteins, resistance, wild cultivars, and Peruvian."

The focus of the organization of the information was on the IPM for the blueberry, the same that gave rise to two management proposals: (1) a specific proposal of integrated management of *Chloridea virescens* in the cultivation of blackberries in Peru, based on practices of Cultural Control, Ethological Control, Biological Control, and Chemical Control (PQUA and PBUA). This proposal is also supported by the results of field tests on chemical control PBUA of *Chloridea virescens* in blueberry, carried out in 2018 by the first author of this article, given its implication in the proposed IPM; (2) a proposal for genetic improvement in cultivated varieties of blueberry in Peru, to allow sustainable cultivation of this crop.

DEVELOPMENT

Strengths and Opportunities for blueberries in Peru

According to Guo et al., 2019 and Meiners (2007), the strengths and opportunities are summarized in: (1) Ability to transport high volumes by sea. In 2019, 5 million kg per week were exported; (2) Capacity to produce ten months a year; (3) The US will remain attractive; it is more stable and can absorb large volumes; (4) The Asian market has the possibility of growth and is demanding in terms of presentation, fiber, and sweetness, for which it is expected that "genetics" will help; (5) We have achieved yields of up to 16.8 t/ha even being on a learning curve; (6) There is potential for its reproduction via tissue culture (42 days to 75 days, 75% to 100% rooting).

Phenology and pests of blueberry

Phenology requires to be based on the location of the fruiting stage, that is, on the harvest, to obtain the highest economic returns thanks to the commercial window there is between September and October; for this, pruning is the fundamental activity since it is the starting point in the calendar (Maticorena, 2017). Changing the phenology and forcing not to harvest in other months often implies a more significant presence of pests, and for this, a good IPM program must be designed.

The plant's growth is divided into two phases or stages: vegetative and reproductive. Four stages of vegetative growth are specified, where the first is the vegetative bud, the second is the shoot characterized by short internodes, the third is the lengthening of the internodes and the expansion of the leaves, and the fourth is a new branch made up of fully extended leaves and long internodes (Rivadeneira & Carlazara, 2011). There are six stages of reproductive growth: first, there is a swollen bud that will give rise to the flowers, and later the bud will open, initiating flowering. The third is flower buds with the closed corolla, the fourth is the flower in full bloom with the open corolla, the fifth is the drop of the corolla and fruit set, and the sixth is green fruit (Meyer & Prinsloo, 2003).

Cisternas (2013) mentions the following pests in Chile: White worms (*Hylamorpha elegans, Sericoides spp., S. viridis, S. obesa, Brachysternus prasinus, B. spectabilis, Phytholaema herrmanni, P. dilutipes* and *Tomarus villosus*); Burritos (*Aegorhinus superciliosus*, *Aegorhinus nodipennis, Aegorhinus phaleratus, Otiorhynchus sulcatus, Otiorhynchus rugosostriatus, Naupactus xanthographus, Graphognatus leucoloma* and *Naupactus cervinus*); Thrips (*Frankliniella occidentalis, Thrips tabaci,* and *Frankliniella australis*), Cuncunillas negra (*Dalaca pallens* and *D. variabilis*); Cutworms (*Agrotis ipsilon* and *Peridroma saucia*), Aphids (*Aphis gossypii* and *Macrosiphum* spp.); Leafroller (*Proeulia* spp.); White piglets (*Pseudococcus viburni, P. calceolariae, P. longispinus* and *P. cribata*); Brown fruit bug (*Leptoglossus chilensis*) and shoot borer wasp (*Ametastegia glabrata*).

Integrated Pest Management (IPM) in blueberries

Since the 1940s, entomologists began work related to Integrated Pest Management (IPM) as a "supervised control". The concept of IPM has been changing over time. However, it is still conceived as a crop protection system that integrates pest management techniques (cultural, ethological, biological, chemical, and genetic practices) (Deguine et al., 2021). IPM combines different management strategies and practices to grow and maintain healthy crops while minimizing the use of pesticides. As the cornerstone of sustainable agriculture, it aims to improve farmers' practices to support increased incomes while improving the conservation and management of natural resources and the health of rural communities and consumers. IPM emphasizes the growth of a healthy crop with the least possible disturbance of agroecosystems and encourages natural pest control mechanisms (Food and Agriculture Organization [FAO], 2014).

Among the components of IPM, we have mainly: cultural control, ethological control, biological control, chemical control, and genetic control. Below we detail information about the uses and scientific advances of these components:

Cultural Control: Tasks such as irrigation, fertilization, weed control, and pruning can be cited among the most important. Proper fertilization and irrigation management are essential to ensure high yields and good quality characteristics in the fruit (Baiano et al. 2011). In blueberries, factors included are choosing an appropriate variety for the area, checking the plants in the nursery to avoid weeds, adequate fertilization and irrigation, planting at the correct time, good quality of plants to ensure a good population, a vigorous initial growth, etc.

(Morales, 2017).

Ethological Control: This control method includes physical (light and color) and chemical (semiochemical) factors intended to modify the insect's behavior. The behavior of insects is determined by their response to the presence or occurrence of stimuli of a chemical nature, although they also respond to physical and mechanical stimuli (CARE, 2006). For example, acoustic communication in insects has recently been studied with ethological control applications (Eskov, 2017).

Biologic control: Since the 19th century, 6227 biological control agents (BCA) have been registered, of which 686 have not yet been identified at the species level, that is, there is a knowledge gap for research (Cock, 2019). A series of habitat modifications are included to create conditions to achieve biological control that favors the survival, fertility, longevity, and action of natural enemies and improve their colonization of the crop (Landis et al., 2000). In this context, biological corridors would act as a refuge area for beneficial insects (pollinators and biological controllers) when the conditions in the crop become harsh or deadly due to the applications programmed for pest control (Landis et al. 2000; Gurr et al. 2004).

Chemical Control: They provide quick action and grant a wide range of uses and forms of application (FAO 2002). Among the pesticides for agricultural use, we find Chemical Pesticides for Agricultural Use (PQUA) and Biological Pesticides for Agricultural Use (PBUA). We consider the latter within a chemical control because they come in formulations that include other components in addition to the biological components. For example,in the case of PQUAs, the term includes substances or mixtures of substances applied to crops before or after harvest to protect the product against deterioration during storage and transportation. On the other hand, PBUAs are all the substances of a biological nature: microorganisms or products derived from their metabolism; bacteria, fungi, etc. Likewise, products derived directly from vegetables, which are not chemically synthesized such as: strychnine, nicotine, pyrethrins, rotenone, and garlic, among others, which alone or in combination with adjuvants, are used to prevent, repel, combat, and destroy insects, mites, pathogens, nematodes, weeds, rodents, or other biological organisms harmful to plants, their products, and derivatives (Supreme Decree No. 001-2015-MINAGRI). Both treatments are highly effective in-field pest control (Llanos & Apaza, 2018).

Within the group of bacteria, there are formulations based on *Bacillus thuringiensis* a facultative anaerobic microorganism, chemoorganotrophic, and with catalase activity (Zhou et al., 2020). They can ferment glucose, fructose, trehalose, maltose, and ribose, and hydrolyze gelatin, starch, glycogen, esculin, and N-acetyl-glucosamine (Sauka & Benintende, 2008). However, the main characteristic of *B. thuringiensis* is that during the sporulation process, it produces a parasporal inclusion formed by one or more crystalline bodies of protein nature that are toxic to different invertebrates, especially insect larvae. These proteins are called Cry (from Crystal) and constitute the basis of the most widespread biological insecticide worldwide (Schnepf et al., 1998; Liu et al., 2018).

Bt toxins began to be used commercially in France in 1938; by 1958, their use had spread to the United States. Starting in the 80s, Bt became a pesticide of worldwide interest (Feitelson et al., 1992). Commercialized products of *B. thuringiensis* mainly consist of spore and crystal preparations, activated or not, which are sprayed on crops as if they were conventional insecticides. These preparations generally come from the subspecies kurstaki (Btk) (Kamatham et al., 2021). *B. thuringiensis* is classified into 84 serovars identified by flagellar antigen H serology (Sauka & Benintende, 2008). Since its discovery, some subspecies active, mainly Bt subsp. Kurstaki (Btk), Bt subsp. Thuringiensis (Btt) y Bt subsp. Galleriae (Btg) against pest invertebrates have been reported (Rashki et al., 2021) based on biochemical and morphological characteristics and flagellar antigens (Schnepf et al., 1998).

Crystalline toxins exist in a variety of forms: bipyramidal, spherical, rhomboid, cuboidal, elliptical and irregular, among others, and are active against a large number of groups of insects mainly, as well as nematodes and protozoa (Rashki et al., 2021; Jurat-Fuentes & Crickmore, 2017). The genes that encode these proteins reside in conjugable megaplasmids (Feitelson et al. 1992) were named Cry, and their encoded proteins were designated Cry δ-endotoxins (Ochoa & Arrivillaga, 2009). Cry proteins are synthesized as inactive protoxins ingested by larvae when feeding. The inclusions are solubilized under the alkaline conditions of the larva's digestive tract and are converted by insect proteases into active peptides (Schnepf et al., 1998; Feitelson et al., 1992). The active toxin is recognized by a specific receptor and is inserted into the membrane of the brush border of microvilli of the digestive tract of the insect (Gerber & Shai, 2000). An oligomerization occurs, resulting in the formation of cation channels of 0.5 to 1 nm in diameter (Gerber & Shai, 2000). These pores cause a nonspecific influx of ions, mainly K+ ions, which dissipates ionic gradients and lowers the pH of the medium, causing osmotic cell lysis that leaves the larva unable to feed (Schnepf et al. 1998). On the other hand, the tissue destruction allows the mixture of the contents of the digestive tract with the hemolymph, which, together with the low pH, favors the germination of bacterial spores, leading to septicemia and the death of the larva a few days after ingestion of crystals (Schnepf et al. 1998). When the insect consumes the Cry protein, it presents cessation of ingestion, intestinal paralysis, vomiting, diarrhea, osmotic decompensation, total paralysis, and death (Vachon et al., 2012; Bravo et al., 2007).

In the case of lepidopterans such as *H. virescens*, the proteins considered toxic are those of the Cry1 class. Cry proteins generally show reduced activity spectra and are often limited to a few species of insects belonging to the same order. However, the toxicity of Cry1 proteins is not restricted to lepidopterans (Sauka & Benintende, 2008). For *H. virescens* we have the following toxic proteins: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ae, Cry1Be, Cry1Ca, Cry1Fa, Cry1If, Cry1Ja, Cry1Jc, Cry2Aa, Cry2Ab, Cry2Ac, Cry2Ae, Cry9A, Cry9Ca, and Vip3 (Sauka & Benintende, 2008). *B. thuringiensis var. kurstaki* HD-1 is one of the strains of *B. thuringiensis* best studied and is characterized by carrying the following cry antilepidopteran genes: cry1Aa, cry1Ab, cry1Ac, cry2Aa, cry2Ab and cry1Ia (Sauka, 2007). *B. thuringiensis var. kurstaki* HD-1 is par excellence the strain usedto control of lepidopteran insects, agricultural pests, and forest pests. This strain was initially isolated by Dulmage in 1970, constituting a milestone in the history of the use of *B. thuringiensis* as a larvicide since it was responsible for *B. thuringiensis*-based products can compete with chemical insecticides in terms of efficiency. This strain was up to 200 times more toxic for some species of Lepidoptera than other strains used in the products of that time (Sauka & Benintende, 2008). These products, formulated based on Bts, are used mainly to control lepidoptera pests in corn, wheat, cotton, and fruit crops. Formulations can be made from *B. thuriengensis* isolated from Peruvian agroecosystems and the evaluation of their bioinsecticidal potential.

Flores et al. (2011), managed to isolate 54 strains of *Bacillus thuringiensis* from 385 samples of rhizosphere, plant material, and dead insects from central Peru; the identification of the isolated strains was carried out by observation in phase-contrast microscopy according to the culture microscopic characteristics and differential biochemical tests. Isolated strains were compared with *B. thuringiensis* HD-11, *B. thuringiensis var kurstaki* HD-342, and *B. thuringiensis* aizawai NRRL-HD-130 to evaluate the entomotoxic effect. The Bt-UNMSM-42 strain was the one that presented higher toxicity than the rest of the isolated strains, with mean mortality of 39.73% with 50 µg/mL and 71.93% for 250 µg/mL, with a standard deviation of 11.30 and 9.98, respectively; however, it did not outperform the reference strains *B. thuringiensis* HD-11 and *B. thuringiensis var kurstaki* HD-342, which reached mean mortality of 86.5% and 82.5% respectively at a dose of 250 µg/mL. According to Sauka & Benintende (2008), genetic engineering developed many species of plants that express cry genes from *B. thuringiensis* and thus turned them into "insecticidal plants". These plants are commonly referred to as "Bt plants or crops" (e.g., Bt corn, Bt cotton, etc.). Tobacco plants (*Nicotiana tabacum*) that produced sufficient amounts of Cry protein to control first instar larvae of *Manduca sexta* were developed. Since then, at least ten different types of cry genes have been introduced into 26 plant species: cry1Aa, cry1Ab, cry1Ac, cry1Ba, cry1Ca, cry1H, cry2Aa, cry3A, cry6A, and cry9C (Sauka & Benintende, 2008).

Genetic Control: Varietal resistance is an integrated pest management (IPM) strategy that has been considered an alternative that can be ecological since it can reduce dependence on the use of synthetic insecticides and is compatible with other control methods (Vallejo & Estrada, 2002).

According to Jiménez (2009), genetic control of pests has been used in two ways: (1) The crop can be genetically manipulated to increase its resistance to attack by pests, and (2) Pests can be subject to genetic intervention with the introduction of masses of individuals with a selected genotype. Over the years, varieties of insect-resistant crops have been developed, most notably alfalfa, corn, cotton, beans, cassava, vegetables, rice, sorghum, soybeans, and wheat.

Within Integrated Pest Management, the genetic resistance of a plant to insect attack is a component that can be managed through conventional genetic improvement programs, which in turn are associated with desirable agronomic characteristics (Deguine et al., 2021). These characteristics can be found in the wild varieties of many species, such as the blueberry, where Rodríguez-Saona et al. (2019) found that *D. suzukii* prefers cultivated fruits for oviposition and better hosts for their offspring than wild fruits. The cultivated fruits were also two times larger, 47% firmer, 14% less acidic, and had lower amounts of Brix, phenolic, and anthocyanin per mass than wild fruits.

The review of the scientific literature presented above suggests that through a genetic improvement process we can select those resistance characteristics of wild cultivars to cultivated ones while maintaining other desirable agronomic characteristics directly related to higher crop yield and resistance to plagues and diseases.

Integrated Management Proposal

The proposal developed by the authors regarding the management of *Chloridea virescens* in blueberries is the following:

Cultural Control: The implementation of 6 cultural practices is recommended: (1) Weed control: blueberry leaves are small and few, so the damage of *H. virescens* is significant in the production of this crop. That is why weeds that harbor H. *virescens* egg-laying should be avoided such as Trifolium repens (Fabaeae), species of the genus Geranium (Geraniaceae), and others (Blanco et al., 2008). In addition, geomembranes should be placed throughout the soil of the entire crop area before the blueberry plants are installed to prevent and control the weeds from sprouting and developing.

(2) Management of the environment or field edges: this is where the pest can be harbored; management must be done with evaluations and applications of low impact products on beneficial fauna; (3) Use of windbreaker curtains: for both horizontal and vertical crop management. The wind brings thrips and sand that causes stress to the plantation, making it susceptible to pests and diseases; (4) Management of planting density: in recent years we have gone from sowing 5000 pl/ha to 10,000 pl/ha. Therefore, it is essential to handle high pruning well to avoid ambush of the blueberry, in addition to more control in the evaluations, evaluating a more significant number of plants due to the high density; (5) Use of nets: to prevent the entry of *H. virescens* in the field. The use of nets reduces the entry of 80% of lepidopterans (6) Know the neighboring crops: to project the influence and management in the field.

Ethological Control: The following are recommended: (1) Use of molasses and light traps: for *H. virescens*, ten molasses traps should be used per hectare; (2) Pheromone use: the dose for *H. virescens* is 10 to 15 pheromones per ha. They must be placed from the beginning to the end of the campaign. The pest population must be evaluated weekly and correlated with the captures in pheromones.

Biological Control: At least four techniques are recommended: (1) Biological corridors: planting the biological corridors around the field and changing them 3 to 4 times a year. The goal is to have flowers throughout the year because they are a food source of pollen and nectar for predators such as chrysopids and ladybugs, species like fennel, yarrow, sage, alder, buttercup, and sunflower; (2) Bedbugs: concerning predatory bedbugs, biological corridors will be planted to maintain them and guarantee their action of preying eggs and larvae of *H. virescens;* (3) Chrysopids: As for chrysopids, a minimum of 30 thousand per hectare should be released every 15 days and 2 to 3 releases should be made in a row, always with prior evaluation. The larvae need prey which are the eggs and larvae of H. *virescens*. Adults need nectar and pollen; for this reason, it is crucial to plant biological corridors; (4) Pollinating bees: since the crop needs pollinators, it is essential to use low-impact products for predators and pollinators.

Chemical Control: Application of PQUAs and PBUAs as follows:

PQUA: Use contact products such as emamectin benzoate or chitin synthesis inhibitors as a last resort.

PBUA: Use nuclear polyhedrosis virus in small larvae and on well-wet foliage, preferably in the afternoon or at night. For this, the use of Bts products is known; the most pathogenic commercial strains must be chosen, with doses ranging from 300 to 700 gr per cyl. Like viruses, they must be applied in the afternoon or at night and are effective in stage III larvae. The larvae are affected after feeding on the first day and will die on the 4th-6th day. Bt continues to be a powerful tool for controlling *H. virescens* larvae as there is no resistance.

This proposal is based on organic agriculture's technological and profitability challenges (Dilas-Jiménez et al., 2020).

Proposal for genetic improvement for durable or quantitative resistance in cultivated varieties of blueberries

The genetics of resistance to pests by crops is under the control of two types of genes: (1) Vertical resistance, of a specific race, of significant effects and not durable; it is controlled by one or a few genes, and its inheritance is qualitative; (2) Horizontal resistance, of non-specific race, of minor genes and long-lasting; it is controlled by multiple genes, each of which contributes to resistance and its inheritance is quantitative.

Quantitative resistance is the one we choose in this work for being durable and can be used by traditional methods with the support of modern selection techniques. However, before developing the genetic improvement proposal for quantitative resistance in blueberries, we believe it is necessary to point out the characteristics of the techniques that we will propose to use, and we will explain why we will not use other techniques even though it is believed "that they are the most indicated."

This proposal follows these steps:

Identification of resistance genes

It is known that all cultivated varieties of blueberries are susceptible to damage by *H. virescens*; therefore, a collection of wild plant material related to the cultivated blueberry will be carried out; it will then be evaluated for resistance, and those with good response will be selected. Native varieties of wild relatives are a pool of genes from which economic resources are generated by developing improved varieties.

Some of the phenotypic characteristics that should be evaluated are (1) Hardness of the leaf in view that in the field, it is seen that they are slightly attacked by *H. virescens*, (2) Concentration of the fruit in the upper part so that this pest does not have many organs at disposals such as buds and flowers, in addition to being able to program today's scarce personnel in the fields, (3) The distance between the buds so that *H. virescens* does not have many microclimates below the middle third of the plant. Experience: The United States, through the United States Department of Agriculture (USDA), the Agricultural Research Service (ARS), and the National Clonal Germplasm Repository (NCGR) maintain a gene bank in Oregon with more than 1800 accessions of *Vaccinium spp*. which come from 34 countries (Bassil et al., 2020).

Incorporation of genes from the wild relative to the cultivated varieties

The samples (accessions) selected in the previous step will be hybridized with the cultivated ones. Experience: Allotetraploid hybrids derived from the crossing of *Vaccinium uliginosum* and *Vaccinium vitis-idaea* are fertile, thus offering genetic variability from which to select many characteristics, such as yield, fruit quality, and adaptation to variable ecological conditions in the breeding of *V. vitis-idaea* (Morozov, 2007).

Selection - backcross - selection, assisted by modern techniques

In the hybrids derived from the previous cross, the plants (F1) that have genes with quantitative effects will be selected through modern techniques, which will be backcrossed (F1 X cultivated) with the cultivated varieties to recover the fruit quality genes of the cultivated ones and will be re-selected in the progeny of the backcross until identifying plants with resistant quantitative trait QTLs genes. Conventional genetic improvement for quantitative resistance has given good results (Kolmer, 1996); however, it has drawbacks. First, an extensive group of individuals needs to be evaluated to find the quantitative resistance genes together; in addition, hundreds of plants have to be inoculated or infested. These are delicate activities, and it is not always possible to inoculate or infest homogeneously in the field. Modern techniques appear as a powerful tool in enhancing the selection of these types of characters. Experience: The use of molecular markers in the genetic improvement of plants is proven. Garkava-Gustavsson et al. (2005) used RAPID and ISSR markers to assess the genetic diversity of 15 mountain cranberry *Vaccinium vitis-idaea* populations, 13 from Sweden, Finland, Norway, Estonia, and Russia, and two populations of *V. minus* from Japan and Canada. Genetic differentiation between accessions can be exploited in hybridization programs of this species (Garkava-Gustavsson et al. 2005). Marker-assisted selection (MAS) -for quantitative inheritance traits- is being applied in breeding programs and directed pyramiding in different crops (Liu et al. 2004; Asea et al. 2009; Moloney et al. 2010; Singh et al. 2005). Single nucleotide polymorphisms (SNP) generated by genotyping by sequencing (GBS) allowed identifying QTLs of additive effect for resistance to Fusarium in wheat (Zhang et al., 2020). In cotton, with the same technology, 3187 polymorphic markers were developed, which allowed the identification of 17 quantitative trait loci (QTL) for the height of the plant, the height of the first fruiting branch node and the number of vegetative shoots (Qi et al. 2017). Similarly, four QTLs for resistance and one QTL for susceptibility to leaf rust in alfalfa were identified in the genetic map in an alfalfa mapping population (Adhikari & Missaoui, 2019).

Clonal propagation (asexual) of the selected plants

Plants selected by modern techniques will be multiplied asexually; they will be taken to field trials with large plots. Experience: One of the most significant advantages of the genus Vaccinium is that it responds well to asexual propagation both by in vitro methods and cuttings.Blueberry species such as *V. corymbosum, V. virgatum* and *V. macrocarpon*, they root up to 76% in medium without growth regulators (Tetsumura et al. 2017 and Debnath and McRae 2011 cited by Erst et al. 2018). In turn, Erst et al. 2018 specify that most plants require specific chemicals for the initiation of cell differentiation and the formation of meristems, which is why, in its study by *in vitro* methods, a rooting of up to 100% in blueberry variety “Golubaya rossyp” was achieved. Guo et al. (2019), using a rooting bag method, obtained 97.7% rooting after using the blueberry cultivar ‘Ozarkblue’ in a culture medium for woody plant supplemented with 0.1 mg/l of IBA; likewise, they achieved densities of 1600 seedlings per m² compared to the traditional rooting method that manages to put 270 to 420 seedlings per m² (Guo et al. 2019). In micropropagation *in vitro*, transgenic plants of *V. corymbosum* and *V. vitis-idaea had* a better response in the regeneration of shoots using zeatin at a concentration of 20μM due to its effect on the induction of regeneration of adventitious shoots from cut leaves. It is also specified that *in vitro* they can be easily rooted using IBA or *ex vitro* in a humidity chamber without hormonal treatment (Meiners et al. 2007).

Identification of the best clone with quantitative resistance

After vegetative multiplication, the plants selected by modern techniques will be taken to the field with natural and artificial inoculation; at the end of the trial, the best material will have been identified, which would be the improved variety.

Why did we not choose CRISPR-Cas?**:** To apply this technique, it is required that the quantitative resistant genes have been identified first; likewise, hundreds of genes would have to be edited at the same time, which makes it almost impossible use of this technique nowadays in the case of genetic improvement in blueberry; also, it is still in the process of refining, so it is not safe. Experience: The CRISPR-Cas system, discovered as an immune system acquired by certain bacteria, seems to have notable advantages in gene editing over ZFN and TALEN as they are potent tools (Ran et al. 2013; Chen et al. 2019). In the yeast *Pichia pastoris,* a CRISPR/Cas9 system was developed with episomal sgRNA plasmid and 100% genome editing efficiency was obtained, as well as high multicopy gene editing and stable multigene editing without a substantial decrease caused by multi-sgRNA (Yang et al. 2020). However, off-target DNA cleavage remains one of the major imperfections of the system, including sequence mutation, rearrangement, activation, and cell death during genome editing (Chen et al. 2020).

Why do we not recommend Bt transgenic with Cry proteins? Effects of resistance to Bt transgenics: The main characteristic of Bt is the production of protein crystals containing toxins with specific activity against many pests, including dipteran, lepidopteran and coleopteran insects, as well as nematodes, protozoa, trematodes, and mites (Adalat et al. 2020). The gene variants of Cry toxins obtained from the bacterium *B. thuringiensis* (Bt), due to their insecticidal effect, have become an alternative to chemical insecticides in agriculture (Zhou et al. 2020; Grove et al. 2001; Zhang et al. 2018; Zhang et al. 2020, because they control pests of lepidoptera (moths) and coleoptera (beetles) that feed on plants. Two bacterial isolates –variants of Cry toxins-, controlled the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), with mortality rates of 100 and 96.6% (Abo-Bakr et al., 2020). Another chimeric protein toxin involving CryIA residues 450-612, demonstrated 30 times more activity against *H. virescens* than the native parental toxin, indicating that this region plays an essential role in the specificity of *H. virescens* (Ge et al. 2020). However, they are ineffective for sap-sucking insects (Hemiptera) (Liu et al. 2020). Susceptible insects acquire resistance in a few years, and many new strains of Bt have been isolated to avoid resistance to pests (Zhou et al., 2020).

The widespread cultivation of transgenic soybeans has caused significant changes in the spectrum of lepidopteran larvae, both in the number of species and their densities in the field. Transgenic crops that produce insecticidal toxins from *B. thuringiensis* (Bt) have successfully reduced the incidence of the most common caterpillars that infest soybeans, such as *Anticarsia gemmatalis* (Lepidoptera: Erebidae) and *Chrysodeixis includens* (Lepidoptera: Noctuidae). However, lepidopteran species not previously registered have been found in cultivation due to the possibility of adaptation to genetically modified cultivars. For example, the appearance of *Peridroma saucia* Hübner (Lepidoptera: Noctuidae) is described for the first time in Brazil, feeding on genetically modified soybean cultivars (Takahashi et al. 2019). After five years of research , Downes et al. (2010), , found a significant exponential increase in the frequency of alleles that confer resistance to Cry2Ab in Australian field populations of *Helicoverpa punctigera*, since the adoption of a Bt cotton; in addition, the frequency of alleles of resistance to the cry2Ab protein in populations from cultivation areas is eight times higher than those found for populations from regions not cultivated with Bt; a similar result was found for *Diatraea saccharalis* Fabricius (Grimi et al. 2018).

The development of resistance among lepidopterans is a common phenomenon, and a repertoire of resistance mechanisms to various Cry toxins has been identified from a laboratory, greenhouse, and field studies in this insect

(Peterson et al. 2017). Cases of pest resistance to crystal proteins Bt (Cry) produced by transgenic crops increased from 3 in 2005 to 16 in 2016 (Tabashnik et al., 2017). Gassmann (2016) found that in laboratory selection experiments, the western corn rootworm could develop resistance to all types of Bt corn after three to seven generations of selection. The "pyramids" of transgenic crops that produce two or more toxins of *B. thuringiensis* (Bt) active against the same pest are used to delay the evolution of resistance in insect pest populations (Santos-Amaya et al., 2015). However, this strategy could fail if a single gene in a pest confers resistance to many toxins, as happened with the CP73 strain of the cotton pest *H. virescens* (F.), which is resistant to the Cry1Ac and Cry2Aa toxins of Bt (Gahan et al. 2005). All the blueberry varieties grown in Peru are introduced. Unfortunately, no work has been done to evaluate resistance to *Chloridea virecens,* and it seems that they are susceptible since this pest has been found in all of them; however, resistance genes will be donated by wild relatives collected in Peru, if a genetic improvement program is developed.

In 2005, seven years after releasing a transgenic Bt variety of maize resistant to *Busseola fusca* (Lepidoptera noctuidae), significant levels of pest survival were observed (Van den Berg et al. 2013). Under laboratory conditions, Gassman (2016), showed that between three and seven generations of selection, the pest *Diabrotica virgifera* could generate resistance to all types of Cry proteins. In Australia, a population of *Helicoverpa armigera* developed resistance to the Cry1Ac toxin from *B. thuringiensis* because around 70% of resistant larvae *H. armigera* were able to survive on Cry1Ac transgenic cotton (Gunning, 2005).

FINAL COMMENTS

The integrated management of blueberries, especially the PBUA chemical control with products based on Bt proteins Cry, and the biological control are highly explicitly recommended for controlling *Chloridea virescens*.

The proposal for genetic improvement of varieties of good yield and acceptable quality but susceptible constitutes a good strategy in the medium and long term. Using native varieties would allow the possibility of accumulating genes of lasting resistance for local pests in the susceptible ones. With modern selection techniques, this activity would be more efficient and results in a shorter time than what would be obtained with the traditional method alone.

Conflicts of interest

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations that could unduly influence this manuscript.

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Elaboration and execution, Development of methodology, Conception and design; Editing of articles and supervision of the study have involved all authors.

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References

Abo-Bakr, A., Fahmy, E. M., Badawy, F., Abd El-latif, A. O., & Moussa, S. (2020). Isolation and characterization of the local entomopathogenic bacterium, *Bacillus thuringiensis* isolates from different Egyptian soils. *Egyptian Journal of Biological Pest Control, 30*(1), 1–9.

Adalat, R., Saleem, F., Crickmore, N., Naz, S., & Shakoori, A. R. (2017). In vivo crystallization of three-domain Cry toxins. *Toxins, 9*(3), 80.

Adhikari, L., & Missaoui, A. M. (2019). Quantitative trait loci mapping of leaf rust resistance in tetraploid alfalfa. *Physiological and Molecular Plant Pathology, 106*, 238–245.

Agrodata Perú. (2020a). *Arándanos Perú Exportación* 2019-diciembre. https://www.agrodataperu.com/2020/01/arandanos-peru-exportacion-2019-diciembre.html

Agrodata Perú. (2020b). *Arándanos: ¿por qué si el Perú es el primer exportador en el mundo aún no conquista nuestra mesa?* https://www.agrodataperu.com/2020/03/arandanos-peru-primer-exportador-mundial.html

Asea, G., Vivek, B. S., Bigirwa, G., Lipps, P. E., & Pratt, R. C. (2009). Validation of consensus quantitative trait loci associated with resistance to multiple foliar pathogens of maize. *Phytopathology, 99*(5), 540–547.

Bassil, N., Bidani, A., Nyberg, A., Hummer, K., & Rowland, L. J. (2020). Microsatellite markers confirm identity of blueberry (*Vaccinium* spp.) plants in the USDA-ARS National Clonal Germplasm Repository collection. *Genetic Resources and Crop Evolution*, 1–17.

Blanco, C. A., Terán-Vargas, A. P., Abel, C. A., Portilla, M., Rojas, M. G., Morales-Ramos, J. A., & Snodgrass, G. L. (2008). Plant host effect on the development of Heliothis virescens F. (Lepidoptera: Noctuidae). *Environmental Entomology*, *37*(6), 1538–1547.

Bravo A., Gill S.S., Soberón M. (2007). Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon 49*: 423–435.

Buzeta, A. (1997*). Chile: Bayas para el 2000*. Fundación Chile 133 p. Concepción, facultad de Agronomía. Chile.

CARE Perú. (August, 2006). *Manejo integral de plagas - Guia para pequeños productores agrarios*. Lima Perú, s.e.

Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual review of plant biology, 70*, 667–697.

Chen, S., Yao, Y., Zhang, Y., & Fan, G. (2020). CRISPR system: Discovery, development and off-target detection. *Cellular Signalling, 70*, 109577.

Cisternas A., Ernesto. (2013). Insect pest of economic importance associated with blueberry. Cap. 8 *Blueberry Manual.* Chile.

Cock, M. J. (2019). Unravelling the status of partially identified insect biological control agents introduced to control insects: an analysis of BIOCAT2010. *BioControl, 64*(1), 1–7.

Contreras-Pérez, M., Hernández-Salmerón, J., Rojas-Solís, D., Rocha-Granados, C., del Carmen Orozco-Mosqueda, M., Parra-Cota, F. I., ... & Santoyo, G. (2019). Draft genome analysis of the endophyte, *Bacillus toyonensis* COPE52, a blueberry (*Vaccinium spp*. var. Biloxi) growth-promoting bacterium. *3 Biotech, 9*(10), 1–6.

Deguine, J. P., Aubertot, J. N., Flor, R. J., Lescourret, F., Wyckhuys, K. A., & Ratnadass, A. (2021). Integrated pest management: good intentions, hard realities. A review. *Agronomy for Sustainable Development, 41*(3), 1–35

Dilas-Jiménez, J. O., & Cernaqué, O. (2017). *El sector cafetalero peruano: Un enfoque a la CTI para su competitividad*. Universidad Continental.

Dilas-Jiménez, J., Zapata-Ruiz, D., Arce-Almenara, M., Ascurra-Toro, D., & Mugruza-Vassallo, C. (2020). Análisis comparativo de los costos de producción y rentabilidad de los cafés especiales con certificación orgánica y sin certificación. *South Sustainability, 1*(2), e017.

Downes, S., Parker, T., & Mahon, R. (2010). Incipient resistance of *Helicoverpa punctigera* to the Cry2Ab Bt toxin in Bollgard II® cotton. *PLoS One, 5*(9), e12567.

Erst, A. A., Gorbunov, A. B., & Erst, A. S. (2018). Effect of concentration, method of auxin application and cultivation conditions on in vitro rooting of bog blueberry (*Vaccinium uliginosum* L.). *Journal of Berry Research, 8*(1), 41–53.

Eskov, E. K. (2017). The diversity of ethological and physiological mechanisms of acoustic communication in insects. *Biophysics, 62*(3), 466–478.

Food and Agriculture Organization. (2002). *Manual Práctico - Manejo Integrado de Plagas y Enfermedades en cultivos hidropónicos en invernadero*. s.l., s.e.

Food and Agriculture Organization. (2014). *The international code of conduct on pesticide management*.

Febres, F. (2013). Resultados en Arándano deben ser vistos con serenidad. *Revista Red Agrícola* no. 11, 6–9.

Feitelson J. S.; Payne J., & Kim L. (1992). *Bacillus thuringiensis*: insects and beyond. *Nat. Biotech. 10*, 271–275.

Flores, A., Alcarraz, M., Woolcott, J. C., Benavides, E., Godoy, J., Huerta, D., ... & Patiño, A. (2011). Biodiversidad de *Bacillus thuringiensis* aislados de agroecosistemas peruanos y evaluación del potencial bioinsecticida. *Ciencia e Investigación*, *14*(1), 30–35.

Gahan, L. J., Ma, Y. T., MacgregorCoble, M. L., Gould, F., Moar, W. J., & Heckel, D. G. (2005). Genetic basis of resistance to Cry1Ac and Cry2Aa in *Heliothis* *virescens* (Lepidoptera: Noctuidae). *Journal of economic entomology, 98*(4), 1357–1368.

García Rubio, JC; Gonzáles de Lena, G; Ciordia Ara, M. (2018). *El cultivo del arándano en el norte de España*. Asturias, España, s.e. [19 jul. 2020]. http://www.serida.org/pdfs/7452.pdf

Gargurevich, G. (2017). Biloxi ¿la red globe de los arándanos? *Revista Red agrícola, 39*(1), 24-26.

Garkava-Gustavsson, L., Persson, H. A., Nybom, H., Rumpunen, K., Gustavsson, B. A., & Bartish, I. V. (2005). RAPD-based analysis of genetic diversity and selection of lingonberry (*Vaccinium vitis-idaea* L.) material for ex situ conservation. *Genetic Resources and Crop Evolution, 52*(6), 723–735.

Gassmann, A. J. (2016). Resistance to Bt maize by western corn rootworm: insights from the laboratory and the field. *Current opinion in insect science, 15*, 111–115.

Gerber D., & Shai Y. (2000). Insertion and organization within membranes of the δ-endotoxin pore-forming domain, helix 4-loop-helix 5, and inhibition of its activity by a mutant helix 4 peptide. *J. Biol. Chem., 275*, 23602–23607.

Gestión. (July, 2019). Producción de arándanos en Perú crece 796% más que hace cuatro años, pero su precio en chacra cae | Economía (on line)). https://gestion.pe/economia/arandanos-produccion-minagri-produccion-de-arandanos-en-peru-crece-796-mas-que-hace-cuatro-anos-pero-su-precio-en-chacra-cae-noticia/?ref=gesr

Grove, M., Kimble, W., & McCarthy, W. J. (2001). Effects of individual *Bacillus thuringiensis* insecticidal crystal proteins on adult *Heliothis virescens* (F.) and *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). *BioControl, 46*(3), 321–335.

Grimi, D. A., Parody, B., Ramos, M. L., Machado, M., Ocampo, F., Willse, A., ... & Head, G. (2018). Field-evolved resistance to Bt maize in sugarcane borer (*Diatraea saccharalis*) in Argentina. *Pest management science, 74*(4), 905–913.

Gunning, R. V., Dang, H. T., Kemp, F. C., Nicholson, I. C., & Moores, G. D. (2005). New resistance mechanism in *Helicoverpa armiger*a threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin. *Applied and environmental microbiology, 71*(5), 2558–2563.

Guo, Y. X., Zhao, Y. Y., Zhang, M., & Zhang, L. Y. (2019). Development of a novel in vitro rooting culture system for the micropropagation of highbush blueberry (*Vaccinium corymbosum*) seedlings. *Plant Cell, Tissue and Organ Culture (PCTOC), 139*(3), 615–620.

Gurr, G. M., Wratten, S. D., Tylianakis, J., Kean J., & Keller M. (2004). *Providing Plant Foods for Insect Natural Enemies in Farming Systems: Balancing Practicalities and Theory*, in F.L.

Hancock, J. (2009). Producción de arándano Alto. *Agronomijas Vēstis*, (12), 35–38.

Jiménez, EM. (July, 2009). *Métodos de Control de Plagas*. Managua, Nicaragua, s.e. https://cenida.una.edu.ni/relectronicos/RENH10J61me.pdf

Jurat-Fuentes, J. L., & Crickmore, N. (2017). Specificity determinants for Cry insecticidal proteins: Insights from their mode of action. *Journal of invertebrate pathology*, *142*, 5–10.

Kamatham, S., Munagapati, S., Manikanta, K. N., Vulchi, R., Chadipiralla, K., Indla, S., & Allam, U. S. (2021). Recent advances in engineering crop plants for resistance to insect pests. *Egypt J Biol Pest Control,* 31, 120.

Kolmer, J. A. (1996). Genetics of resistance to wheat leaf rust. *Annual review of phytopathology, 34*(1), 435–455.

Landis, D., Wratten, S., & Gurr, G. (2000). Habitat management to conserve natural enemies of arthropod pests in Agriculture. *Annual review of entomology, 45*, 175–201. https://doi.org/10.1146/annurev.ento.45.1.175

Liu, B., Zhang, S., Zhu, X., Yang, Q., Wu, S., Mei, M., ... & Leung, H. (2004). Candidate defense genes as predictors of quantitative blast resistance in rice. *Molecular Plant-Microbe Interactions, 17*(10), 1146–1152.

Liu, Y., Wang, Y., Shu, C., Lin, K., Song, F., Bravo, A., ... & Zhang, J. (2018). Cry64Ba and Cry64Ca, Two ETX/MTX2-type Bacillus thuringiensis insecticidal proteins active against hemipteran pests. *Appl. Environ. Microbiol., 84*(3), e01996-17.

Llanos, A., & Apaza, W. (2018). Antifungal activity of five chemical and two biological fungicides for the management of Botrytis cinerea, causal agent of Gray Mold in Strawberry. *Peruvian Journal of Agronomy, 2*(1), 1–8

Ministerio de Desarrollo Agrario y Riego. (August, 2020). *El arándano en el Perú y en el mundo- Producción, Comercio y Perspectivas. Lima. Perú.* Pág. 8.

Meiners, J., Schwab, M., & Szankowski, I. (2007). Efficient in vitro regeneration systems for Vaccinium species. *Plant Cell, Tissue and Organ Culture, 89*(2-3), 169–176.

Meyer, H. J. & Prinsloo N. (2003). Assessment of the potential of blueberry production in South Africa.

Small Fruits Review, 2, 3–21.

Moloney, C., Griffin, D., Jones, P. W., Bryan, G. J., McLean, K., Bradshaw, J. E., & Milbourne, D. (2010). Development of diagnostic markers for use in breeding potatoes resistant to *Globodera pallida* pathotype Pa2/3 using germplasm derived from *Solanum tuberosum* ssp. andigena CPC 2802. *Theoretical and applied genetics, 120*(3), 679–689.

Morales, C. G. (2017). Manual de manejo agronómico del arándano (on line). Chile, s.e. [2 jul. 2020]. https://www.indap.gob.cl/docs/default-source/default-document-library/manual-arandanos.pdf?sfvrsn=0

Morozov, O. V. (2007). The Prospects for Using *Vaccinium uliginosum* L.× *Vaccinium vitis-idaea* L. Hybrid in Breeding. *International journal of fruit science, 6*(4), 43–56.

Ochoa, G., & Arrivillaga, J. (2009). *Bacillus thuringiensis*: Avances y perspectivas en el control biológico de *Aedes aegypti*. *Boletín de Malariología y Salud Ambiental, 49*(2), 181–191.

Qi, H., Wang, N., Qiao, W., Xu, Q., Zhou, H., Shi, J., ... & Huang, Q. (2017). Construction of a high-density genetic map using genotyping by sequencing (GBS) for quantitative trait loci (QTL) analysis of three plant morphological traits in upland cotton (*Gossypium hirsutum* L.). *Euphytica, 213*(4), 83.

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature protocols, 8*(11), 2281–2308.

Rashki, M., Maleki, M., Torkzadeh-Mahani, M., Shakeri, S., & Nezhad, P. S. (2021). Isolation of Iranian Bacillus thuringiensis strains and characterization of lepidopteran-active cry genes. *Egyptian Journal of Biological Pest Control*, 31(1), 1–10.

Rivadeneira, M., & Carlazara G. (2011). *Comportamiento fenológico de variedades tradicionales y nuevas de arándanos*. Instituto Nacional de Tecnología agropecuaria. Argentina.

Rodríguez-Saona, C., Cloonan, K. R., Sanchez-Pedraza, F., Zhou, Y., Giusti, M. M., & Benrey, B. (2019). Differential susceptibility of wild and cultivated blueberries to an invasive frugivorous pest. *J Chem Ecol.45*(3).

Santos-Amaya, O. F., Rodrigues, J. V., Souza, T. C., Tavares, C. S., Campos, S. O., Guedes, R. N., & Pereira, E. J. (2015). Resistance to dual-gene Bt maize in *Spodoptera frugiperda*: selection, inheritance, and cross-resistance to other transgenic events. *Scientific reports, 5*, 18243.

Sauka, D. (2007). *Estudio de genes y proteínas insecticidas de aislamientos nativos de Bacillus thuringiensis. Aportes al conocimiento de su distribución y toxicidad en plagas agrícolas*. [Doctoral dissertation, UBA].

Sauka, D. H., & Benintende G. B. (2008). *Bacillus thuringiensis*: generalidades. Un acercamiento a su empleo en el biocontrol de insectos lepidópteros que son plagas agrícolas. *Revista Argentina de Microbiología, 40* (2), 124–140.

Schnepf E., Crickmore N., Van Rie J., Lereclus D., Baum J., & Feitelson J. (1998). Bt and its pesticidal cristal proteins. Microbiol. *Mol. Biol. Rev. 62,* 775–806.

Sierra Exportadora. (2011). *Perfil Comercial-Arándano Deshidratado. Asociación Regional de Exportadores de Lambayeque*. Área de Comercio Exterior.

Sierra Exportadora – Presidencia del Consejo de Ministros de Perú (PCM). (2012). *Estudio de prefactibilidad para la producción y comercialización de arándanos* (*Vaccinium corymbosum* L.) en condiciones de valles andinos. Estudio elaborado por Ing. Liliana Benavides. 146 pp.

Singh, R. P., Huerta-Espino, J. U. L. I. O., & William, H. M (2005). Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turkish Journal of Agriculture and Forestry, 29*(2), 121–127.

Tabashnik, B. E., & Carrière, Y. (2017). Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology, 35*(10), 926.

Takahashi, T. A., Nishimura, G., Carneiro, E., & Foerster, L. A. (2019). First record of *Peridroma saucia* Hübner (Lepidoptera: Noctuidae) in transgenic soybeans. *Revista Brasileira de entomologia, 63*(3), 199–201.

Peterson, B., Bezuidenhout, C. C., & Van den Berg, J. (2017). An overview of mechanisms of Cry toxin resistance in lepidopteran insects. *Journal of Economic Entomology, 110*(2), 362–377.

Vachon V., Laprade R., & Schwartz J. L. (2012). Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: A critical review. *J. Invertebr. Pathol*. In press.

Vallejo, F., & Estrada, E. (2002). *Mejoramiento genético de plantas.* [Universidad Nacional de Colombia]. DIPAL. Palmira, Colombia. 404 p.

Van den Berg, J., Hilbeck, A., & Bøhn, T. (2013). Pest resistance to Cry1Ab Bt maize: Field resistance, contributing factors and lessons from South Africa. *Crop Protection, 54*, 154–160.

Yang, Y., Liu, G., Chen, X., Liu, M., Zhan, C., Liu, X.,

& Bai, Z. (2020). High efficiency CRISPR/Cas9 genome editing system with an eliminable episomal sgRNA plasmid in Pichia pastoris. Enzyme and *Microbial Technology, 138.* 109556.

Zhang, P., Guo, C., Liu, Z., Bernardo, A., Ma, H., Jiang, P., ... & Bai, G. (2020). Quantitative trait loci for Fusarium head blight resistance in wheat cultivars Yangmai 158 and Zhengmai 9023. *The Crop Journal, 9*(1), 143–153.

Zhang, X., Gao, T., Peng, Q., Song, L., Zhang, J., Chai, Y., ... & Song, F. (2018). A strong promoter of a non-cry gene directs expression of the cry1Ac gene in *Bacillus thuringiensis*. *Applied microbiology and biotechnology, 102*(8), 3687–3699.

Zhou, Y., Wu, Z., Zhang, J., Wan, Y., Jin, W., Li, Y., & Fang, X. (2020). *Bacillus thuringiensis* novel toxin Epp is toxic to mosquitoes and *Prodenia litura* larvae. *Brazilian Journal of Microbiology*, 1–9.